Identification of Squamous Cell Carcinoma Susceptibility Genes Using Homozygous Mapping

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by

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ABSTRACT

Cutaneous squamous cell carcinoma (SCC) is a type of non-melanoma skin cancer. SCC is the second most common skin tumor with a lifetime risk of 7-11 percent. It is a neoplasm of epidermal keratinocytes characterized by loss of cellular structure and architecture. The molecular pathways leading to the development of SCC remain poorly defined. Loss of heterozygosity (LOH) in tumors is a frequent event contributing to tumorigenesis. Homozygous loci have two identical alleles or DNA sequences, and a higher frequency of genomic homozygosity has been observed in normal DNA from cancer patients compared to controls. Thus, there may be an increased likelihood that stretches of consecutive homozygous markers, or runs of homozygosity (ROH), may occur at loci containing cancer susceptibility genes. Based on these observations, our first hypothesis is that ROH are more frequent in normal DNA from individuals with SCC. Secondly, we hypothesize that certain single nucleotide polymorphisms (SNPs) are associated with risk of SCC. To test these hypotheses, DNA from SCC patients and controls were genotyped at 123 SNPs mapping to Chromosomes 7 and 11 by Sequenom Mass ARRAY. We observed similar frequencies of homozygosity in cancer and non-cancer patients and no significant ROH were identified. One SNP on chromosome 7 and two SNPs on chromosome 11 had significant odds ratios (OR) for SCC risk in the homozygous state. Three SNPs mapping to *Hdac9* on chromosome 7 and five SNPs mapping to *DRD2* and *KIRREL3* on chromosome 11 show protective effects. From these data, we conclude that some SNPs on chromosome 7 and 11 may increase the risk of developing SCC and others promote protection. We found *Hdac9, DRD2,* and *KIRREL3* to be potential candidate genes that affect SCC susceptibility. Additional studies are needed to confirm these results and identify skin cancer susceptibility and resistance genes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Biology of the Skin

Skin cancer is the most common human cancer and is typically classified as melanoma skin cancer or nonmelanoma skin cancer (NMSC). Melanoma arises from the malignant growth of the melanocytes, skin cells that produce the pigment melanin, and NMSC originates in the external surface and epithelial layers of the skin. One in five individuals will develop NMSC in their lifetime, and the chance of developing this disease is greater than that of any other type of tumor[1]. Economically, skin cancer places a significant pressure on health care, resulting in treatment costs of over $500 million annually in the United States[2].

NMSC is further divided into basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), both of which originate from malignantly transformed epidermal keratinocytes in the basal and squamous cell layers respectively[3]. BCC is the most frequent skin tumor and makes up about 80% of diagnosed cases (www.cancer.org). SCC is the second most frequent skin tumor with a lower lifetime risk (7-11%) in comparison to BCC (18-33%)[4]. This study will focus on SCC.

SCC tumorigenesis is often described as a multistep process affecting the squamous (flat, scale-like) cells that make up the most superficial layer of the epithelium[5]. Actinic keratoses (AK), also known as solar keratoses (SK), are well-established sun-induced precancerous lesions that progress to SCC *in situ*, a continuation of keratinocyte dysmaturation and atypical cell structure and formation. SCC *in situ* is characterized histologically by the abnormal development of keratinocytes throughout the full epidermis, representing a preinvasive stage of SCC[6]. Keratoacanthomas (KA) are benign cutaneous squamous neoplasms that also arise from UV exposure and share similarities with SCC in growth rate and morphology. It is currently

being debated as to whether or not KAs are part of the development of SCC. SCC is described as a neoplasm of epidermal keratinocytes characterized by loss of cellular structure and architecture.

The major environmental contribution to the development of human skin cancers is UV light through sun exposure. Despite the benefits of UV, such as its role in vitamin D synthesis and stimulating the production of skin pigmentation, UV is a complete carcinogen that is capable of promotion, initiation, and progression of skin cancer. It causes photodamage and other negative effects of the skin. For instance, UV radiation can result in mutations such as DNA nucleotide dimers, the peroxidation of lipids, and protein crosslinking[7]. There is an abundance of evidence that UV exposure is a contributor to skin cancer. In animal models, UV radiation has been shown to be sufficient enough to produce skin cancer in mice[8]. Consistent with this, the development of skin cancer occurs at a higher frequency at sites that are exposed to UV, such as the head and neck. Consequently, the rates of developing skin cancer are low in rarely UV-exposed areas like the buttocks. Chronic exposure to UV radiation also predisposes an individual to NMSC. Additionally, individuals with the genetic disorder xeroderma pigmentosum (XP) are deficient in repairing UV-induced DNA damage and are therefore more likely to develop skin cancer in sun exposed areas without protective measures. Lastly, individuals with sensitive skin that burns easily, such as those with fair skin, are more susceptible to the development of skin cancer[9].

1.2 Genetics of Skin Cancer

As of today, the exact molecular pathways that convert normal epidermal cells to SCC remain poorly defined. However, there have been number of studies on the genetic changes that

occur in skin cancer tumorigenesis. UV light has the potential to induce somatic (non-heritable) DNA mutations. The most investigated of these aberrations is mutation-associated inactivation of the tumor suppressor gene *p53*. *p53* is important in DNA repair, as it regulates the cell cycle and triggers apoptosis (programmed cell death) of damaged cells to prevent their proliferation. Not only is *p53* a hotspot for mutations, but differential expression of p53 response elements have been observed[10]. For example, keratin 15 (*KRT15*) is a p53 response element that is downregulated in SCC, thus affecting the integrity of cellular architecture. Abnormal keratinocyte differentiation in SCC is possibly caused by the disruption of gene activation and/or repression during the differentiation cycle. Chronic UV radiation and *p53* mutations, specifically C → T transitions, can result in the dysregulation of apoptosis and the abnormal proliferation of keratinocytes. AK lesions, considered to be precursors to SCC, contain a high frequency of *p53* mutations that are also found to be present in SCCs[11].

Currently, there is little known about specific genetic risk factors and susceptibility to SCC. However, a few studies have suggested genetic predispositions to the disease. In a Swedish population-based study, a family-shared environmental effect of 17-18% was found[12]. This shared effect may be partially caused by familial habits of sun exposure. SCC was found to have a genetic contribution of 8% and a childhood-shared environmental contribution of 7%. Previous studies of genetic contributors to this disease have found an increased relative risk of 2.7 in individuals who have a family member with SCC compared to individuals with no family history of SCC. Inherited traits within families, such as skin type and pigmentation, can also lead to the common sensitivity to UV radiation and an increased risk for skin cancer.

One common feature in cancer cells is genomic instability, which is caused by either inherited mutations in genes important for genome integrity or mutations acquired in somatic

cells during tumor development[13]. These genetic alterations can occur at the level of single nucleotides, small stretches of DNA, whole genes, or complete chromosomes and their structures. Specific to SCC development, chromosomal aberrations such as gains, losses, and translocations have been identified[14]. SCCs experience high chromosomal instability, and a number of these have been and are being studied today to find genes implicated in this disease. The investigation for these genetic changes in SCC and other cancers has led to the development of various genomic studies and findings.

1.3 Genomic Studies

1.3.1 Single Nucleotide Polymorphisms and Alleles

With the completion of the Human Genome Project, it is now possible to use genome-wide association studies (GWAS) to identify susceptibility genes. GWAS are used to detect genetic differences between genomes of individuals with and without disease. These studies rapidly scan specific markers across a genome to find common genetic variations that are associated with a particular phenotype (disease). Identifying these differences can help elucidate the possible genetic contributions to disease. Genetic markers that are often used in GWAS are single nucleotide polymorphisms (SNP). A SNP is a DNA variation at a single nucleotide position in the genome. SNPs can potentially affect gene regulation or later protein production by altering mRNA and the protein reading code. Changes in the genome can affect the predisposition one has towards a disease, and can alter the function of susceptibility genes and other genes that confer resistance.

SNP mapping technologies like SNP genotyping arrays are used to detect polymorphisms and determine genotypes. These technologies have made it possible to study chromosomal

aberrations in more detail. By using mapping technologies, deviations from what is considered genetically normal can be measured.

Genotypes reflect the genetic makeup of an individual and typically consist of two alleles, one inherited from each parent. In normal cells, an individual has two copies of DNA, each called an allele, from every region in the genome with the exception of the X and Y chromosomes. Sometimes these regions share the same version of a SNP (homozygous) and sometimes they have different versions (heterozygous).

1.3.2 Allelic Imbalance and Loss of Heterozygosity

SNP genotyping arrays have been used as a tool to identify candidate genes, which are genes believed to influence phenotype expression in a way that confers risk to a disease, such as cancer. GWAS such as genotyping arrays are important tools for describing the genetic events that underlie the cause and progression of cancer. One genome-wide allelic imbalance study found deletions within the tyrosine phosphatase receptor type D (*PTPRD*) on chromosome 9 in tumors. PTPRD is associated with metastasis and is a possible candidate tumor suppressor gene for cutaneous SCC[15].

SNP genotyping arrays are also used to study allelic imbalance, a differential and altered expression in copy number between two alleles. Alleles are alternate forms of a gene at a specific position on the chromosome, such as a nucleotide. Allelic imbalance is the loss or gain in genetic material and is detected by measuring allele or nucleotide frequencies. Normally, an individual has two copies of DNA. However in tumors, changes can occur that result in losses or gains of DNA.

One type of allelic imbalance that has been studied in cancer tissues is loss of heterozygosity (LOH)[16]. A loss of heterozygosity is the loss of one allele and its normal function. LOH is a common form of mutation in cancer, where its presence suggests the loss of a functional tumor suppressor gene. In cancer, the Knudson 2-hit Hypothesis is an explanation of LOH in tumors. This concept encompasses both germline predispositions and somatic mutation events to inactivate tumor suppressors. Therefore, it applies to heritable cancer syndromes. The first hit is an inherited mutant allele in the germline. Although one allele is mutated, the other is a normal, wild-type allele. With the second hit, there is a somatic loss of the wild-type allele. As a result, a single mutant allele is expressed, thereby altering the normal functions of the cell and beginning the process of tumorigenesis. The second hit may be caused by a variety of complex somatic events.

Recent studies have shown that LOH is common at cancer susceptibility loci. A heterozygous locus consisting of a wild-type (cancer resistant) allele and a mutant (cancer susceptible) allele loses its resistant allele, resulting in the expression of its susceptibility allele. Mechanisms by which LOH occurs include deletions, amplification and over-expression of the mutant/susceptibility allele, and mitotic recombination (Figure 1).

1.3.3 Runs of Homozygosity

A related and newly described concept to LOH is runs of homozygosity (ROH), which is currently being studied as a possible contributor to disease. A ROH is a long continuous stretch of homozygous alleles or SNPs in the genome (Figure 2). Researchers studying schizophrenia developed an analytic approached to examining SNPs called whole-genome homozygosity association (WGHA) [17]. WGHA leads to the identification of ROH that are associated with

disease. By applying WGHA to a case-control dataset for the psychological disorder, Lencz et al. found a greater frequency of ROH in schizophrenia patients than in the normal population. Nine ROHs were identified as potentially significant 'risk' factors for this disease. ROH have also been observed in other diseases including autism and Alzheimer's disease[18].

Given that some regions of homozygosity stretch for a relatively long distance of DNA and the presence of numerous tumor suppressor genes in the genome, there is an increased likelihood that stretches of consecutive homozygous markers occur at loci containing cancer susceptibility genes. Homozygous loci can arise by gene conversion during carcinogenesis, and have previously been associated with cancer. It is possible that ROH contributes to an increased cancer risk and predisposition. One study reported the identification of 114 loci with increased frequencies of heterozygosity in genomic (normal) DNA of breast and prostate cancers, and head and neck SCC in comparison to matched controls. Regions of homozygosity have also been found in normal tissues of colorectal cancer patients[19]. Therefore, homozygosity is an important genetic factor that influences cancer predisposition.

1.4 Identification of SCC Susceptibility Loci

Results from Dr. Amanda E. Toland's research lab have provided some background on SCC susceptibility loci by utilizing the divergence of *Mus spretus* from *M. musculus* in mouse studies. *M. spretus* is resistant and *M. musculus* is susceptible to chemically-induced skin cancer. To identify loci for skin cancer susceptibility, backcrosses between the two species were created. *Skin tumor susceptibility 5 (Skts5)*, located on mouse chromosome 12, was identified as a susceptibility locus. Sequencing of genes and coding elements that mapped to the peak linkage area for *Skts5* for each of the different cross-strains was conducted to identify skin cancer

susceptibility candidate genes[20]. The *Skts5* region on mouse chromosome 12 maps to two regions on human chromosome 7 (Figure 3).

Additional studies to map loci important for SCC were also conducted in the Toland lab. One study conducted genotyping of human SCC tumors and matched genomic DNA using microsatellite markers to detect loci showing preferential allelic imbalance (losses and gains) (Dworkin et al. submitted). This study found multiple chromosomal regions showing preferential allelic imbalance and identified significant markers for these imbalances on chromosome 11 (Figure 4). A number of SNPs on 11q23-11q24 were identified to show strong evidence for preferential allelic loss. Specifically, 11q24 showed significant somatic preferential allelic imbalance, and this locus was identified as a new candidate tumor susceptibility locus for SCC (Dworkin, A.M. personal communication).

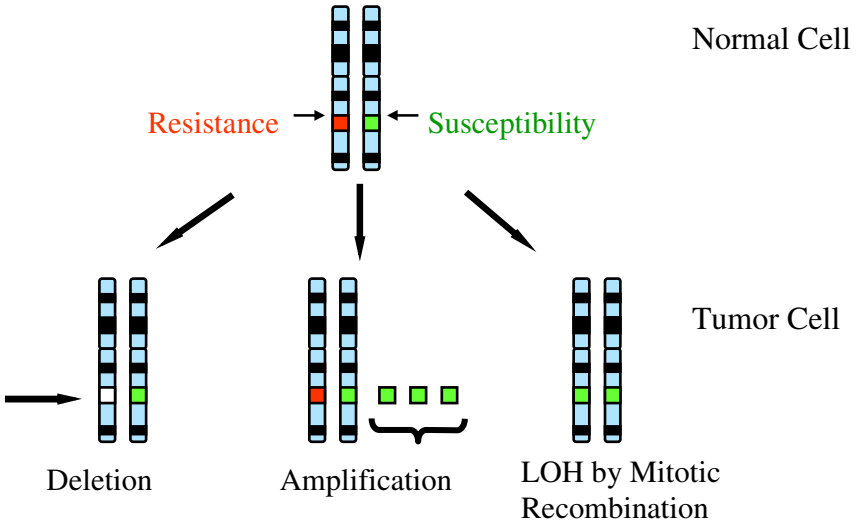

Figure 1: Loss of Heterozygosity (LOH). In cancer, a heterozygous locus on sister chromatids has one resistance allele (red) and one susceptible allele (green). LOH occurs when the heterozygous locus loses its functional resistance allele, resulting in only the susceptible allele being present. LOH can occur by multiple mechanisms including deletion of the resistance allele, amplification of the susceptibility allele, or by mitotic recombination.

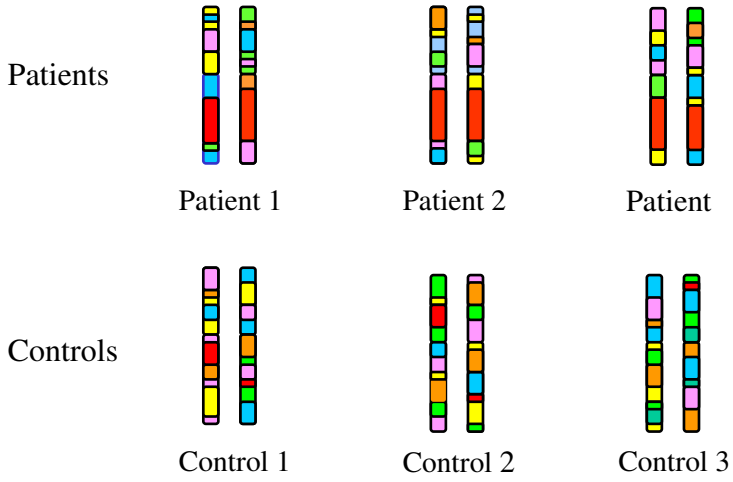

Figure 2: Runs of Homozygosity (ROH). Homozygous genotypes are those with two identical alleles (two identical copies of DNA). Homozygous alleles are denoted with the same two colors (red) on each sister chromatid. A ROH is the presence of consecutive markers for homozygous alleles that occur throughout a region of DNA. This study hypothesized that normal DNA of cancer patients have ROH (indicated above by large regions of red at the same chromosomal location on both copies of DNA). The control population is hypothesized to not harbor ROH at susceptibility loci.

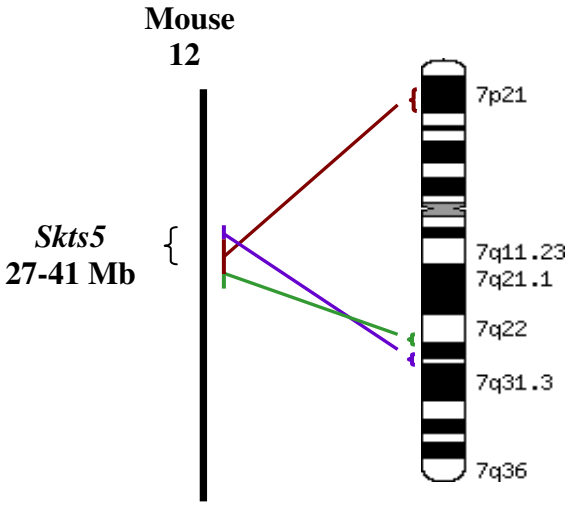

Figure 3: *Skts5* Locus. *Skts5* locus on mouse chromosome 12 has homologous regions to human chromosome 7p21 and 7q31. These areas on human chromosome 7 are of interest in this study.

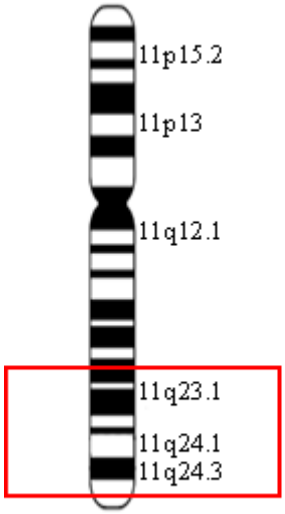

Figure 4: Chromosome 11 Susceptibility Loci. SNPs on 11q23-11q24 were found to exhibit somatic allelic imbalance in SCC tumors, and this region was identified as a new SCC tumor susceptibility locus. These loci are regions of interest in this study.

CHAPTER 2

STUDY RATIONALE AND HYPOTHESIS

Runs of homozygosity, ROH, is a relatively new concept in cancer genetics. Previous studies have found that homozygosity occurs at a higher frequency in normal DNA of various cancers, such as breast and prostate cancer[6]. With the presence of extended homozygous tracts and the numerous cancer susceptibility genes in the genome, there is a high likelihood that ROH will occur at loci of cancer susceptibility genes. Homozygous segments that increase cancer risk can arise from a variety of causes, such as genomic changes that result in recessive mutant cancer genes, biallelic mutations in highly penetrant dominant cancer genes, or low-penetrance cancer predisposition SNPs[7]. One study found an increased frequency of homozygosity at 114 loci in normal DNA from individuals with breast and prostate cancer, and head and neck squamous cell carcinomas. However, ROH has not been studied in cutaneous SCC.

The purpose of this project was to see if this newly described phenomenon of ROH applies to skin cancer. The aim of this study was to determine whether these stretches of homozygosity are more common in normal genomic DNA from individuals with skin cancer than in controls. There are two hypotheses for this project: 1) Runs of homozygosity are common in individuals with skin cancer, specifically squamous cell carcinoma (SCC) and that 2) certain SNPs are associated with risk of SCC. To test these hypotheses, normal DNA from SCC patients (cases) and non-cancer patients (controls) were genotyped at specific SNPs located on chromosome 7 and 11 to look for the presence of homozygosity. The goal of this study was to identify candidate cancer-promoting chromosomes and regions in the genome that contained either ROH or significant SNPs associated with SCC, and to determine the effect of homozygosity on cancer predisposition and development.

CHAPTER 3

MATERIALS AND METHODS

3.1 Patients and Control Samples

Samples in this study consisted of SCC patients (cases) and non-cancer (control) individuals. All patients and individuals signed informed consent. SCC patients were ascertained through OSU Medical Center dermatology clinics. Genomic DNA from both SCC patients and non-cancer patients were used. Collected samples consisted of 185 controls and 277 cases. Controls included in this study were samples in the Columbus-based Controls Collection from the Human Cancer Genetic Sample bank. All controls were age and gender matched to cases, and the average age was 66 years for both sample groups.

3.2 Genotyping

We quantitatively genotyped DNA from both case and control samples using MassARRAY Iplex gold genotyping technology from Sequenom. Sequenom uses a primer oligo base extension assay with matrix-assisted laser desorption ionization time-of-flight mass spectrometry. This technology allows for multiplex analysis of many polymorphisms at once and uses a quantitative approach to determine genotypes.

This SNP genotyping assay is based on a polymerase chain reaction (PCR), followed by a single base primer extension reaction. Forward and reverse primers flanking SNPs are used first in a PCR to amplify the DNA. Extension primers are then used to differentiate and determine the two alleles, or nucleotides, at each SNP. These primers anneal to the DNA adjacent to the SNP, and through a single base extension step, an extra nucleotide complementary to the SNP is added. Since nucleotides differ in mass, the DNA fragments can therefore be separated by mass

using mass spectrometry. The proportion of nucleotides at each SNP was determined and used to indicate a homozygous or heterozygous state. SNP genotypes composed of both Allele 1 and Allele 2 are heterozygous, and SNP genotypes with two copies of Allele 1 or Allele 2 are homozygous. When Sequenom detects only one mass for the DNA fragments, it indicates that the alleles are the same and the genotyping call is homozygous. When Sequenom detects two different masses within the DNA fragments, it indicates that the alleles are different and the genotyping call is heterozygous (Figure 4).

RealSNP Sequenom designer software was used to design nucleotide primer sets and multiplexing mixes for SNPs tagging in genes on chromosomes 7 and 11 (Supplemental Table 1 and 2). Chromosome 7 and 11 were chosen for this study because previous results in the lab suggested that they house SCC susceptibility genes and are implicated in disease progression. Chromosome 7 maps to *Skts5* in mouse and chromosome 11 shows preferential allelic imbalance in SCC tumors. A total of 123 SNPs mapping to chromosome 7 and 11 were genotyping by Sequenom Mass ARRAY (Table 1). Fifty-one SNPs mapped to chromosome 7 and 72 SNPs mapped to chromosome 11. Individual primers were designed for each SNP of interest.

3.3 Sequencing

Following genotype analysis, a number of SNPs were out of Hardy-Weinberg equilibrium. DNA sequencing was conducted to further evaluate the genotyping results for 14 of these SNPs. The Sequenom primers were sequenced to test the presence of biases, such as DNA mutations or polymorphisms in the DNA complementary to the Sequenom Primers. This could affect the primer's ability to bind to the DNA sequence around the SNP. PCR primers were designed to sequence the Sequenom primer binding regions at 14 different SNPs on chromosome

7 and 11 (Supplemental Table 3). PCR was performed using 15ng of input DNA using Taq DNA polymerase (Qiagen, Valencia, California), 1μM forward and reverse primers, 1x Q-solution (Qiagen, Valencia, California), 1x PCR buffer (Quiagen, Valencia, California), and 500μM dNTPs (Denville Scientific, Metuchen, New Jersey) in a final volume of 20μl for 35 cycles at 58ºC. PCR product were run on a 1% agarose gel and visualized by ethidium bromide staining.

PCR products were treated with Exo/SAP-iT to remove single stranded DNA (USB, Cleveland, OH). Automated sequencing of PCR products was conducted on an ABI 3700 by standard methods through the OSU Nucleic Acids Shared Resource. Forward and reverse sequences were analyzed using SeqMan/DNAstar 3.0 (www.dnastar.com) to identify mutations and polymorphisms.

3.4 Statistical Analysis

One aim of this study was to look at the distribution of alleles and genotypes within the case and control population, and to determine their adherence to Hardy-Weinberg Equilibrium (HWE). This principle states that both allele and genotype frequencies in a population remain constant, unless disturbances occur that influence this equilibrium. Constant frequencies depend on random mating, no mutation, no migration or emigration, large population size, and no natural selection. This is an ideal population state that provides a way to measure genetic changes in an affected or diseased population.

This study analyzed the observed genotypes of cancer and non-cancer patients with expected genotypes. We wanted to determine whether the sample population was consistent with or deviated from HWE (Figure 5). Similar observed and expected genotype frequencies are

considered to be within HWE, and varying observed and expected genotype frequencies represented a deviation from HWE. Testing for HWE deviations was performed by a chi (χ^2) square analysis. A p-value for each SNP was determined for significance ($p<0.01$). All genotype data from Sequenom were denoted as observed genotype frequencies (Supplemental Table 4-7). From these, the allele frequencies were calculated and used to determine the expected genotype frequencies under HWE. This was done by using the Hardy-Weinberg equations. Comparison of the observed and expected genotype frequencies can reveal any deviations from HWE. We expected the controls to stay within HWE and the cases (diseased) to deviate from equilibrium.

To address our first hypothesis of the presence of ROH, the percentage of homozygous genotypes at each SNP was calculated. We compared the percent heterozygosity and homozygosity for both the cases and controls, and looked at the SNP locations to identify the presence of ROH. In this study, a ROH was defined as a stretch of at least 12 or more consecutive SNPs with high frequencies of homozygous genotypes.

Our second hypothesis was that certain SNPs are associated with SCC risk. The odds ratio (OR), a statement of the likelihood of an event, and a 95% confidence interval (CI) were computed for each SNP. An OR was calculated for both homozygous and heterozygous genotypes. Upper and lower confidence intervals for the OR were also analyzed for significance. Significant ORs with confidence intervals greater or less than 1.0 indicate association between genotype and an event. The event in this study is the SCC disease state. An OR of 1.0 implies that an individual has an equal likelihood of developing or not developing SCC. SNP ORs with confidence intervals greater than 1.0 indicate that the genotype causes an increased risk of SCC. ORs of less than 1.0 indicate that the genotype acts as a potential protective factor for SCC.

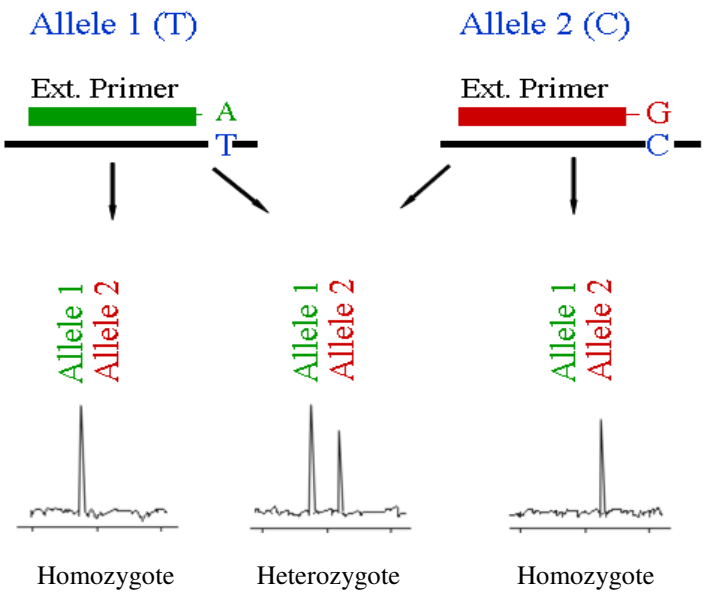

Figure 4: Sequenom iPlex Technology: MassARRAY Iplex gold genotyping technology uses a primer oligo base extension assay and flight mass spectrometry. Based on the differential masses of the alleles (nucleotides) of the SNPs, the genotype can be determined. One peak indicates DNA fragments of the same mass, denoting two identical alleles and a homozygous genotype. Two peaks indicate fragments of differential mass, denoting the SNP as heterozygous.

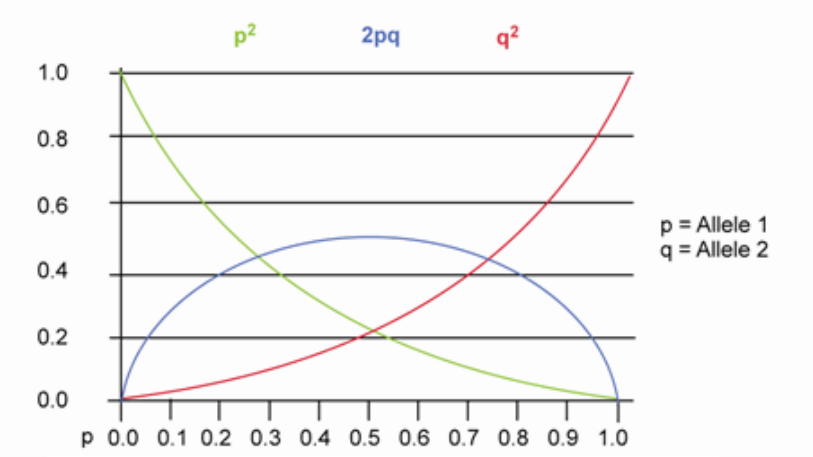

Figure 5: Hardy-Weinberg Equilibrium (HWE): HWE occurs when genotype and allele frequencies remain constant in the absence of disturbing influences. p and q are the frequencies of allele 1 and 2 respectively. p^2 and q^2 are the frequencies of the two homozygotes, and 2pq is the frequency of heterozygotes. Equations are for populations in HWE, and the graph depicts the relationship between the allele and genotype distributions.

Table 1: SNPs of Interest

SNP	Gene	Location
*rs*10225248	Between *ARL4A* and *ETV1*	chr7:13214211
*rs*2214867	Between *ARL4A* and *ETV1*	chr7:13701250
*rs*6461065	Between *ETV1* and *DGKB*	chr7:14072405
*rs*10486048	*DGKB*	chr7:14210927
*rs*2237298	*AHR*	chr7:17314644
*rs*2158041	*AHR*	chr7:17334945
*rs*7811989	*AHR*	chr7:17337888
*rs*2040623	*AHR*	chr7:17347187
*rs*801759	*HDAC9*	chr7:18510723
*rs*11543651	*HDAC9*	chr7:18557184
*rs*801763	*HDAC9*	chr7:18567890
*rs*2520456	*HDAC9*	chr7:18581044
*rs*3807917	*HDAC9*	chr7:18583439
*rs*2520458	*HDAC9*	chr7:18589454
*rs*2073973	*HDAC9*	chr7:18591198
*rs*2695029	*HDAC9*	chr7:18593148
*rs*2695027	*HDAC9*	chr7:18598205
*rs*3213615	*HDAC9*	chr7:18600332
*rs*2239926	*HDAC9*	chr7:18603086
*rs*2704284	*HDAC9*	chr7:18612153
*rs*1405618	*HDAC9*	chr7:18616611
*rs*1554774	*HDAC9*	chr7:18627934
*rs*3801986	*HDAC9*	chr7:18631105
*rs*7796078	*HDAC9*	chr7:18639763
*rs*212664	*HDAC9*	chr7:18729642
*rs*17349342	*HDAC9*	chr7:18781998
*rs*7783171	*HDAC9*	chr7:18795321
*rs*12540224	*HDAC9*	chr7:18797014
*rs*6974011	*HDAC9*	chr7:18804988
*rs*12699991	*HDAC9*	chr7:18827285
*rs*2520362	*HDAC9*	chr7:18830424
*rs*2073964	*HDAC9*	chr7:18844675
*rs*6461387	*HDAC9*	chr7:18864830
*rs*12700003	*HDAC9*	chr7:18872391
*rs*10237366	*HDAC9*	chr7:18882084
*rs*17350355	*HDAC9*	chr7:18895028
*rs*11764843	*HDAC9*	chr7:18901835
*rs*10247238	*HDAC9*	chr7:18909429
*rs*726116	*HDAC9*	chr7:18918611
*rs*6969316	*HDAC9*	chr7:18922212
*rs*6947529	*HDAC9*	chr7:18925467
*rs*726805	*HDAC9*	chr7:18929213
*rs*2158768	*HDAC9*	chr7:18936675
*rs*1034805	*HDAC9*	chr7:18944178
*rs*2853552	*HDAC9*	chr7:18946792
*rs*10486329	*HDAC9*	chr7:18960717
*rs*17140399	*HDAC9*	chr7:18965129
*rs*7808451	*HDAC9*	chr7:18969411
*rs*17140423	*HDAC9*	chr7:18974409
*rs*6968777	*HDAC9*	chr7:18977874

*rs*2526633	*HDAC9*	chr7:18997276
*rs*2587550	Between *ANKK1* and *DRD2*	chr11:112778135
*rs*6276	*DRD2*	chr11:112786607
*rs*2734839	*DRD2*	chr11:112791450
*rs*4245147	*DRD2*	chr11:112823217
*rs*4938023	Between *DRD2* and *TMPRSS5*	chr11:112880057
*rs*4534613	Between *DRD2* and *TMPRSS5*	chr11:112891253
*rs*12361261	Between *DRD2* and *TMPRSS5*	chr11:112891829
*rs*7115090	Between *DRD2* and *TMPRSS5*	chr11:112920930
*rs*11214677	Between *DRD2* and *TMPRSS5*	chr11:112982291
*rs*4471464	Between *DRD2* and *TMPRSS5*	chr11:113009292
*rs*1509513	Between *DRD2* and *TMPRSS5*	chr11:113010123
*rs*12802646	Between *DRD2* and *TMPRSS5*	chr11:113047669
*rs*7933647	Between *DRD2* and *TMPRSS5*	chr11:113055939
*rs*11214716	*ZW10*	chr11:113123185
*rs*2459976	*ZW10*	chr11:113148203
*rs*6589396	Between *ZW10* and *AX748073*	chr11:113151087
*rs*1713676	*AX748073*	chr11:113165786
*rs*7111825	*USP28*	chr11:113201863
*rs*11214741	*USP28*	chr11:113223807
*rs*2097078	Between *USP28* and *HTR3b*	chr11:113258726
*rs*6589400	Between *USP28* and *HTR3b*	chr11:113272935
*rs*1176746	*HTR3*b	chr11:113307811
*rs*17626940	Between *HTR3*a and *ZBZTB16*	chr11:113397918
*rs*238935	*ZBZTB16*	chr11:113471635
*rs*238914	*ZBZTB16*	chr11:113489319
*rs*238903	*ZBZTB16*	chr11:113493997
*rs*763857	*ZBZTB16*	chr11:113502545
*rs*1997547	*ZBZTB16*	chr11:113507954
*rs*2106234	*ZBZTB16*	chr11:113513263
*rs*648181	*ZBZTB16*	chr11:113552800
*rs*655988	*ZBZTB16*	chr11:113577575
*rs*371267	*ZBZTB16*	chr11:113608225
*rs*612592	*RPUSD4*	chr11:125579034
*rs*676982	*RPUSD4*	chr11:125580429
*rs*624590	*FAM118B*	chr11:125588547
*rs*695077	*FAM118B*	chr11:125593159
*rs*11220409	*FAM118B*	chr11:125601174
*rs*17656	*SRPR*	chr11:125638392
*rs*499205	*SRPR*	chr11:125639829
*rs*638766	*SRPR*	chr11:125639908
*rs*667627	*FOXRED1*	chr11:125652907
*rs*591163	Between *FOXRED1* and *TIRAP*	chr11:125653642
*rs*8177374	*TIRAP*	chr7:125668053
*rs*8177376	Between *TIRAP* and *DCPS*	chr11:125668822
*rs*1786704	Between *TIRAP* and *DCPS*	chr11:125670967
*rs*648710	*DCPS*	chr11:125681586
*rs*586566	*DCPS*	chr11:125704828
*rs*240537	*DCPS*	chr11:125718301
*rs*7937122	*AK096370*	chr11:125729658
*rs*11220473	*ST3GAL4*	chr11:125776848
*rs*582037	*ST3GAL4*	chr11:125783285
*rs*2230279	*ST3GAL4*	chr11:125783285

*rs*752806	Between *ST3GAL4* and *KIRREL3*	chr11:125798449
*rs*7925175	*KIRREL3*	chr11:125844645
*rs*1943528	*KIRREL3*	chr11:125882665
*rs*9971527	*KIRREL3*	chr11:125894275
*rs*612841	*KIRREL3*	chr11:125914444
*rs*625496	*KIRREL3*	chr11:125917216
*rs*6590224	*KIRREL3*	chr11:126065650
*rs*4935987	*KIRREL3*	chr11:126107596
*rs*10893564	*KIRREL3*	chr11:126131899
*rs*7127398	*KIRREL3*	chr11:126133006
*rs*1946050	*KIRREL3*	chr11:126162173
*rs*11220621	*KIRREL3*	chr11:126182338
*rs*1106804	*KIRREL3*	chr11:126194653
*rs*7110377	*KIRREL3*	chr11:126231423
*rs*10750355	*KIRREL3*	chr11:126261121
*rs*10893604	*KIRREL3*	chr11:126275543
*rs*1628588	*KIRREL3*	chr11:126293260
*rs*1793668	*KIRREL3*	chr11:126298028
*rs*2508557	Between *KIRREL3* and *PRR10*	chr11:126377995
*rs*7941136	*PRR10*	chr11:126379370

CHAPTER 4

RESULTS

This project is a case-control study that focuses on the genetics of squamous cell carcinoma. Three different ideas and concepts were analyzed for the regions on chromosome 7 and 11. The first was to determine the frequencies of homozygosity and heterozygosity in genomic DNA from cases and controls. We hypothesized that we would find lower frequencies of heterozygosity (and consequently higher frequencies of homozygosity) in the case population compared to controls. From this, we determined if SNPs with high homozygosity levels occurred in a consecutive sequence that would indicate the presence of ROH. Secondly, we were interested in looking at Hardy-Weinberg Equilibrium and comparing the genotype and allele distribution in both the case and control sample populations. Lastly, we wanted to identify SNPs that were associated with increased risk or resistance to the disease by calculating the OR for homozygotes and heterozygotes. An OR greater than one indicates that the SNP has a role in SCC susceptibility, and an OR less than one indicates that the SNP has a protective and resistant effect.

Two rounds of genotyping were conducted. In the first round, 123 SNPs (51 from chromosome 7 and 72 from chromosome 11) were genotyped to gather preliminary results and identify any significant trends in the data. Ten SNPs from chromosome 7 and 20 SNPs from chromosome 11 were then chosen for a second round of genotyping in additional samples to increase sample size and validate previous results. These SNPs were chosen because they exhibited significant HWE p-values in the case population and significant OR suggesting SCC risk and protection. We wanted to see the trends from the initial round of genotyping to continue in a larger sample size and to reach statistical significance for risk. The second round of

genotyping was also done to validate the experimental method and confirm the consistency of genotype calls made by Sequenom.

4.1 Chromosome 7

4.1.1 Initial Genotyping Studies

We initially genotyped 96 control and 172 case DNA samples by Sequenom at 51 SNPs on chromosome 7 to identify regions of ROH and SNPs that deviated from Hardy-Weinberg Equilibrium in the case population (Table 2). The SNPs map across a region of 112 Mb of DNA. The average percent heterozygosity across all SNPs was 39.93% and 42.46% within the case and control samples respectively. Overall frequencies of heterozygosity were similar, and only a few SNPs exhibited significant differences between the cases and controls. The largest ROH we found contained 16 adjacent SNPs mapping to a 120 kb region. Interestingly, no significant and unique ROH was identified in the case population for this region.

A few SNPs exhibited differential genotype distribution between the two populations. The patient population was significantly out of HWE in ($p<0.01$) at *rs*1269991 and the control population deviated from HWE ($p<0.01$) at *rs*2237298. Three of the 51 SNPs (5.88%) were calculated to have an OR for increased risk in the homozygous genotype state, while one of these three also showed increased risk for SCC in the heterozygous state (*rs*3807917, *rs*2695029, *rs*3213615). One SNP was identified to be protective in the heterozygous state (*rs*2520458), and one SNP showed resistance to SCC in the homozygous state (*rs*6974011). Despite the insignificant differences in average percentage heterozygosity between the cases and controls across all of the tested SNPs, one region of interest was identified. A 17kb region containing

four SNPs exhibited significant varying genotype and allele frequencies, heterozygosity and homozygosity, or ORs for risk or protection between the cases and controls.

4.1.2 Validation Genotyping for Selected SNPs

Ten SNPs were genotyped in a total of 220 cases and 138 controls for validation of initial results. Of the interesting SNPs chosen for further genotyping, four SNPS continued to exhibit significant characteristics (*rs*3807917, *rs*2520458, *rs*6974011, *rs*1269991) (Table 3). The average percent heterozygosities for these SNPs were 31.98% in the cases and 35.41% in the controls. Collectively across chromosome 7, there was no significant difference between heterozygosity between cases and controls, although individual SNP differences were observed. The case population exhibited genotypic deviations from HWE ($p<0.01$) at one SNP (*rs*1269991).

Only one SNP, *rs*3807917, had an OR > 1 (OR: 2.30, CI: 1.11-4.74, $p=0.01$), indicating risk in the homozygous state of TT. With additional control and case samples, the percent heterozygosities were 43.48% (50/115) in the cases and 53.62% (74/138) in the controls.

The SNPs *rs*2520458 and *rs*1269991 were calculated to have OR < 1 for heterozygotes. This suggests that having a heterozygous genotype at these SNPs is protective of SCC. Interestingly, for *rs*6974011, both the OR for heterozygotes TG (OR: 0.40, CI: 0.17-0.94) and homozygotes GG (OR: 0.36, CI: 0.16-0.83) were below 1. The 'G' allele acts in a dominant manner, and appears to confer resistance to SCC whenever it is present, either in a heterozygote or homozygous state.

Four SNPs appear to be validated and trends seen from initial genotyping were reinforced. *rs*3807917 continued to have an OR > 1 (CI: 1.11-4.74), suggesting increased risk

for SCC in the homozygous state, and *rs*2520458 continued to exhibit OR < 1 (CI: 0.34-0.89), indicating protection in the heterozygous state. With additional samples, *rs*12699991 had a significant HWE p-value in the case population and was protective in the heterozygous state. These SNPs are located in *Hdac9*, suggesting this gene to be involved in SCC development and susceptibility.

4.2 Chromosome 11

4.2.1 Initial Genotyping Studies

Seventy-two SNPs on chromosome 11 were initially genotyped by Sequenom in 148 case and 35 control DNA samples (Table 4). All SNPs together extend across 114 Mb of DNA. The average percent heterozygosity of the SNPs was 33.57% and 39.79% in the case and control samples respectively. Percent heterozygosities were fairly similar in both the cases and controls, and only a few SNPs exhibited noticeable differences between populations. The longest ROH in the cases contained 20 adjacent SNPs mapping to a 220kb region. However, no ROH were identified to be significant in the case population because similar regions of homozygosity were observed in the control population.

A number of SNPs snowed noticeably different patterns of percent heterozygosity between cases and controls. The case population was out of HWE ($p<0.01$) at ten SNPs (13.89%) and the control population deviated from HWE ($p<0.01$) at four SNPs (5.55%). Four SNPs (5.55%) were calculated to have significant OR for increased risk in the homozygous state (*rs*2734839, *rs*624590, *rs*591163, *rs*752806). The SNP *rs*591163 also exhibits increased risk in the heterozygous state. Other SNPs associated with increased risk in the heterozygous state are *rs*586566 and *rs*240537. Four SNPs (5.55%) were calculated to have significant OR for

resistance in the homozygous state (*rs*1176746, *rs*676982, *rs*638766 and *rs*1793668). Five SNPs (6.94%) had significant ORs for resistance in the heterozygous state (*rs*12361261, *rs*752806, *rs*7127398, *rs*1793668, *rs*7941136).

4.2.2 Validation Genotyping for Selected SNPs

Twenty SNPs on chromosome 11 were chosen for further genotyping in 277 cases and 185 controls. The majority of them exhibited some sort of significant characteristic, including genotype distributions out of HWE ($p<0.01$), or significant OR for risk in either heterozygotes or homozygotes (Table 5). Similar frequencies of homozygosity and heterozygosity were found in both the cases and controls; the average percent heterozygosity across these 20 SNPs was 34.41% and 40.12% in the case and control population respectively. The case population genotype distribution was out of HWE for eight SNPs ($p<0.01$), and the control population's distribution deviated from HWE at one SNP. Two SNPs (10.53%) show significant OR for increased risk in homozygotes (*rs*2734839 and *rs*752806) and no SNPs showed an increase risk in heterozygotes. Six SNPs (26.32%) showed significant OR for resistance to SCC in heterozygotes (*rs*2587550, *rs*2734839, *rs*1509513, *rs*10893564, *rs*7127398, *rs*1793668). SNP *rs*1793668 was protective in both the heterozygous and homozygous states, and other SNPs associated with SCC resistance in the homozygous state were *rs*1176746 and *rs*11220409.

The SNP *rs*2734839 continued to show similar results as those seen in the first round of genotyping. With a larger sample size, the case population continued to exhibit genotype distributions that deviated from HWE ($p<0.01$). Low rates of heterozygosity (and conversely high rates of homozygosity) were seen at this SNP (16.13% (25/155) in cases and 43.56% (71/163) in controls). Cases had three times more homozygotes (GG) than controls while the

controls had three times more heterozygotes (AG) than the cases (44.52% and 15.95% GG homozygotes in cases and controls respectively). The OR calculations for *rs*2734839 show that the heterozygous genotype confers resistance (OR: 0.38, CI: 0.18-0.84) while the homozygous genotype of GG confers an increased risk of SCC (OR: 2.87, CI: 1.62-5.08).

Fourteen SNPs on chromosome 11 were validated. Many continued to exhibit the same significant trends as in the initial round of genotyping. With the additional case and control samples, other SNPs with $p<0.01$ for HWE and significant OR were noted. Significant SNPs were found within the genes *DRD2* and *KIRREL3*, suggesting these genes as contributors to SCC susceptibility and resistance.

4.3 Sequencing of Sequenom Primers

For some SNPs, the case or control population's genotype and/or allele distribution were significantly out of HWE. A few SNPs exhibited disproportionate distributions of genotypes, where some populations contained little or none of a certain genotype. We hypothesized that these distributions were caused by a possible polymorphisms or mutations within the DNA sequence where the Sequenom primers lay, affecting the accuracy of our results. Primers that hybridize to regions containing polymorphisms may have difficulty annealing. This then can possibly influence the fidelity of polymerization during the amplification or extension step.

To test this hypothesis, we sequenced the DNA regions where the Sequenom primers lay for 14 SNPs. We then compared the DNA sequences to determine if any polymorphisms or mutations are present. We found that the Sequenom extension primer for *rs*801763 lay in a region on chromosome 7 that contained a polymorphism (an AA change to AG), suggesting that this was the reason why the particular SNP was out of HWE (Figure 7). None of the other

primers for the other SNPs out of HWE appeared to contain polymorphism in the samples sequenced; thus we do not have an explanation for the disproportionate genotype distributions.

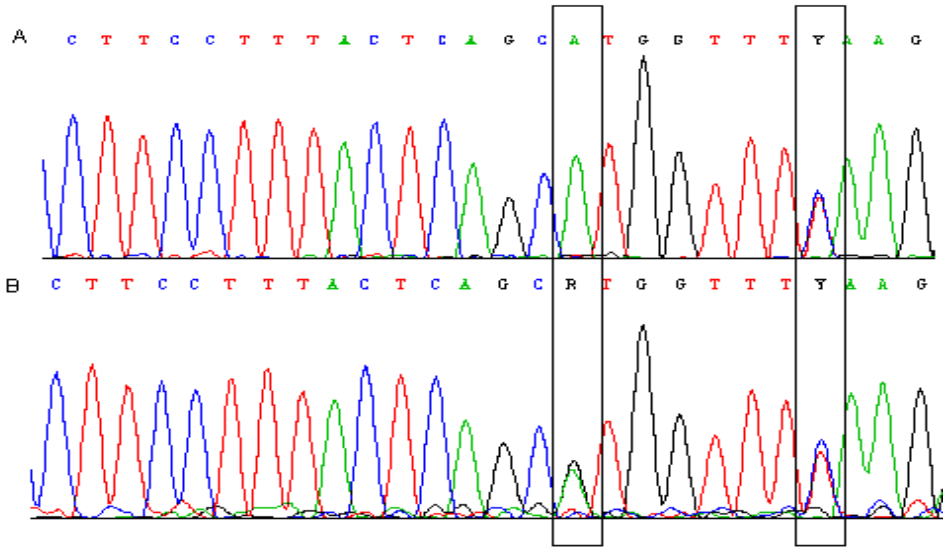

Figure 7: Sequencing of Sequenom Primers. Normal DNA sequence on top and DNA sample from the study on bottom. Extension primer for SNP *rs*801763 lay in a chromosome 7 region containing a polymorphism from AA to AG (left box, R=AG). The SNP is in the right box, Y indicating a genotype of TC.

Table 2: Chromosome 7 Initial Genotyping Results

SNP	# Cases	# Controls	HWE p-value Case	HWE p-value Control	% Het Case	% Het Control	OR Heterozygote (95% CI)	OR Homozygote (95% CI)
*rs*10225248	24	151	0.586	0.810	41.67	50.30	0.61 (0.23-1.56)	0.62 (0.18-2.21)
*rs*2214867	16	130	0.341	0.432	37.50	46.15	0.48 (0.14-1.63)	0.47 (0.12-1.82)
*rs*6461065	87	158	0.749	0.275	31.03	36.71	0.79 (0.45-1.39)	1.36 (0.35-5.26)
*rs*10486048	85	155	0.766	0.911	22.35	19.35	1.27 (0.11-14.95)	1.06 (0.09-11.88)
*rs*2237298*	21	116	0.519	0.008	52.38	27.59	2.06 (0.40-10.70)	0.67 (0.13-3.53)
*rs*2158041*	32	147	0.279	0.445	51.16	25.17	0.99 (0.22-4.56)	0.29 (0.06-1.30)
*rs*7811989	42	150	0.098	0.125	40.48	30.67	2.75 (0.56-13.45)	1.01 (0.20-4.94)
*rs*2040623	85	160	0.114	0.026	24.71	30.63	0.70 (0.38-1.28)	0.54 (0.19-1.57)
*rs*801759*	74	83	0.332	0.645	20.27	9.64	N/A (N/A)	N/A (N/A)
*rs*11543651	18	123	0.130	0.018	22.22	30.89	0.74 (0.12-4.48)	1.18 (0.24-5.88)
*rs*801763*	36	86	0.391	0.774	25.00	27.91	0.82 (0.34-2.00)	0.00 (N/A)
*rs*2520456	87	156	0.586	0.379	34.48	44.23	0.80 (0.27-2.35)	1.23 (0.43-3.54)
*rs*3807917*	22	141	0.011	0.134	22.73	51.77	0.41 (0.13-1.30)	3.43 (1.12-10.50)
*rs*2520458*	85	159	0.087	0.046	35.29	52.20	0.53 (0.30-0.94)	1.35 (0.56-3.24)
*rs*2073973	85	159	0.334	0.934	44.71	49.69	0.68 (0.25-1.32)	0.72 (0.35-1.49)
*rs*2695029*	39	148	0.101	0.749	58.97	48.65	3.83 (1.08-13.62)	3.90 (1.03-14.80)
*rs*2695027	39	146	0.639	0.591	38.46	45.89	0.60 (0.28-1.29)	0.43 (0.13-1.40)
*rs*3213615*	26	153	0.249	0.868	38.46	43.14	1.23 (0.27-3.21)	4.06 (1.30-12.70)
*rs*2239926	87	158	0.647	0.564	40.23	39.87	1.00 (0.31-3.22)	0.98 (0.31-3.11)
*rs*270428 4	86	155	0.463	0.172	53.49	55.48	0.76 (0.40-1.43)	0.65 (0.29-1.43)
*rs*1405618	24	154	0.586	0.503	33.33	44.16	0.71 (0.13-3.74)	1.14 (0.23-5.64)
*rs*1554774	82	153	0.990	0.287	41.46	35.29	1.32 (0.75-2.33)	1.13 (0.42-3.04)
*rs*3801986	21	135	0.505	0.462	57.14	52.59	1.06 (0.31-3.58)	0.80 (0.20-3.27)
*rs*7796078	22	155	0.143	0.238	27.27	39.35	0.57 (0.20-1.58)	0.91 (0.24-3.52)
*rs*212664	41	145	0.103	0.130	29.27	39.31	0.88 (0.28-2.81)	1.50 (0.51-4.43)
*rs*17349342	42	143	0.801	0.371	47.62	51.05	0.58 (0.23-1.47)	0.43 (0.20-1.46)
*rs*7783171	43	149	0.758	0.390	30.23	33.56	0.88 (0.42-1.85)	1.70 (0.30-9.75)
*rs*12540224	85	156	0.554	0.694	52.94	50.64	0.94 (0.46-1.90)	0.77 (0.35-1.69)
*rs*6974011*	15	125	0.447	0.842	40.00	44.00	0.26 (0.07-1.00)	0.17 (0.04-0.71)
*rs*12699991*	85	156	0.008	0.729	34.12	49.36	0.56 (0.31-1.02)	1.18 (0.57-2.45)
*rs*2520362	43	150	0.878	0.048	51.16	58.00	0.86 (0.37-2.00)	1.29 (0.48-3.47)
*rs*2073964	42	153	0.853	0.996	45.24	47.06	0.96 (0.24-2.72)	1.06 (0.27-3.02)
*rs*6461387	20	114	0.257	0.242	35.00	54.39	0.51 (0.13-1.93)	1.19 (0.32-4.41)
*rs*12700003	43	153	0.738	0.942	51.16	49.02	1.01 (0.47-2.15)	0.80 (0.29-2.21)
*rs*10237366	85	155	0.629	0.422	49.41	51.61	1.15 (0.51-2.56)	1.37 (0.59-3.17)
*rs*17350355	19	124	0.161	0.952	31.58	48.39	0.50 (0.13-1.95)	1.02 (0.28-3.72)
*rs*11764843	21	135	0.071	0.405	28.57	45.18	0.47 (0.16-1.39)	0.92 (0.29-2.99)
*rs*10247238	40	148	0.109	0.566	55.00	46.62	2.39 (0.51-11.28)	1.88 (0.39-9.05)
*rs*726116	14	79	0.498	0.706	35.71	40.51	0.87 (0.25-3.01)	1.39 (0.24-7.98)
*rs*6969316	42	153	0.997	0.407	45.24	36.60	0.88 (0.28-2.80)	0.56 (0.18-1.76)
*rs*6947529	17	124	0.597	0.510	41.18	50.81	0.68 (0.22-2.09)	1.02 (0.24-4.41)
*rs*726805	40	144	0.945	0.433	45.00	45.83	0.79 (0.37-1.68)	0.50 (0.17-1.49)
*rs*2158768	41	150	0.183	0.474	39.02	46.67	0.75 (0.31-1.84)	1.05 (0.42-2.63)
*rs*1034805	85	157	0.832	0.500	42.35	47.13	0.81 (0.47-1.42)	0.94 (0.38-2.33)
*rs*2853552	21	147	0.990	0.122	42.86	34.01	1.49 (0.57-3.93)	1.19 (0.23-5.99)
*rs*10486329	81	152	0.575	0.604	53.09	47.37	1.06 (0.53-2.12)	0.74 (0.34-1.61)
*rs*17140399	24	151	0.367	0.804	54.17	46.36	2.14 (0.45-10.18)	1.78 (0.36-8.90)
*rs*7808451	85	155	0.414	0.551	43.53	47.10	0.75 (0.42-1.36)	0.67 (0.32-1.43)

*rs*17140423	41	148	0.071	0.203	31.71	37.16	0.87 (0.40-1.88)	1.60 (0.58-4.41)
*rs*6968777	85	150	0.414	0.496	43.53	45.33	0.94 (0.45-2.00)	1.02 (0.47-2.20)
*rs*2526633	84	156	0.647	0.049	42.86	35.26	2.07 (0.76-5.69)	1.62 (0.60-4.37)

*: SNPs chosen for validation genotyping

Table 3: Chromosome 7 Validation Genotyping for Interesting SNPs

SNP	# Cases	# Controls	HWE p-value Case	HWE p-value Control	% Het Case	%Het Control	OR Heterozygote (95% CI)	OR Homozygote (95% CI)
*rs*2237298	103	127	0.066	0.094	32.04	35.43	1.00 (0.41-2.46)	1.20 (0.51-2.82)
*rs*2158041	141	135	0.758	0.794	39.72	26.67	0.69 (0.20-2.41)	0.36 (0.11-1.20)
*rs*801759	142	110	0.662	0.617	21.83	9.09	0.00 (N/A)	0.00 (N/A)
*rs*801763	129	101	0.625	0.867	25.58	22.77	1.19 (0.64-2.19)	1.65 (0.29-9.27)
*rs*3807917	115	138	0.162	0.189	43.48	53.62	0.92 (0.52-1.64)	2.30 (1.11-4.74)
*rs*2520458	176	137	0.028	0.040	37.50	54.01	0.55 (0.34-0.89)	1.38 (0.64-2.97)
*rs*2695029	134	131	0.305	0.964	43.28	49.62	1.13 (0.59-2.16)	1.81 (0.91-3.60)
*rs*3213615	116	133	0.052	0.257	35.34	45.86	0.42 (0.42-1.22)	1.90 (0.78-4.63)
*rs*6974011	78	127	0.038	0.669	37.18	40.94	0.40 (0.17-0.94)	0.36 (0.16-0.83)
*rs*12699991	176	136	0.001	0.382	35.80	51.47	0.57 (0.35-0.94)	1.30 (0.67-2.52)

Table 4: Chromosome 11 Initial Genotyping Results

SNP	# Cases	# Controls	HWE p-value Case	HWE p-value Control	% Het Case	%Het Control	OR Heterozygote (95% CI)	OR Homozygote (95% CI)
*rs*4938023	69	32	0.766	0.864	44.93	43.75	0.89 (0.24-3.32)	0.80 (0.21-3.01)
*rs*11214677	76	32	0.808	0.837	51.32	50.00	0.72 (0.23-2.27)	0.53 (0.15-1.85)
*rs*2587550*	49	32	0.009	0.656	24.49	40.63	0.53 (0.12-2.27)	1.14 (0.29-4.52)
*rs*6276	76	32	0.230	0.706	32.89	43.75	0.53 (0.21-1.30)	0.41 (0.11-1.52)
*rs*2734839*	56	33	<0.0005	0.305	0.00	54.55	0.00 (N/A)	10.00 (2.49-40.22)
*rs*4245147	60	30	0.611	0.956	46.67	50.00	0.66 (0.21-2.02)	0.59 (0.17-2.04)
*rs*4534613	43	31	0.415	0.686	41.86	45.16	0.96 (0.27-3.43)	1.16 (0.32-4.26)
*rs*12361261*	42	6	<0.0005	0.624	2.38	66.67	0.05 (0.00-0.72)	N/A (N/A)
*rs*7115090	61	29	0.438	<0.0005	44.26	17.24	2.83 (0.85-9.40)	0.52 (0.18-1.51)
*rs*4471464	67	31	0.831	0.366	50.75	58.06	0.87 (0.28-2.68)	1.32 (0.36-4.81)
*rs*1509513*	15	27	0.022	0.795	20.00	51.85	0.24 (0.05-1.22)	1.14 (0.23-5.67)
*rs*12802646	66	33	0.282	0.995	39.39	45.55	0.69 (0.18-2.60)	0.86 (0.23-3.21)
*rs*7933647	8	1	0.719	0.317	37.50	100.00	0.00 (N/A)	N/A (N/A)
*rs*11214716	30	32	0.058	0.355	16.67	28.13	0.56 (0.16-1.91)	N/A (N/A)
*rs*2459976	48	33	0.486	0.127	54.17	33.33	2.70 (0.99-7.39)	1.52 (0.42-5.47)
*rs*6589396	31	30	0.525	0.469	38.71	23.33	2.63 (0.84-8.19)	N/A (N/A)
*rs*1713676*	50	29	0.160	0.015	40.00	62.07	0.00 (N/A)	0.00 (N/A)
*rs*7111825	65	30	0.068	0.584	33.85	40.00	0.78 (0.30-1.99)	1.06 (0.28-3.96)
*rs*11214741	43	29	0.923	0.873	48.84	44.83	0.72 (0.18-2.81)	0.48 (0.12-1.98)
*rs*2097078	69	30	0.286	0.642	34.78	23.33	0.49 (0.05-4.69)	0.25 (0.03-2.14)
*rs*6589400	56	32	0.089	0.192	26.79	37.50	0.00 (N/A)	0.00 (N/A)
*rs*1176746*	37	32	0.558	0.684	43.24	53.13	0.45 (0.16-1.27)	0.16 (0.03-0.94)
*rs*17626940	62	28	0.083	0.423	19.35	28.57	1.00 (0.14-7.39)	1.74 (0.27-11.29)
*rs*238935	66	32	0.982	0.628	33.33	31.25	1.47 (0.21-10.20)	1.37 (0.21-8.84)
*rs*238914	48	31	0.703	0.939	41.67	45.16	1.90 (0.37-9.87)	2.56 (0.50-13.22)
*rs*238903	72	33	0.453	0.396	51.39	48.48	1.33 (0.56-3.17)	2.60 (0.49-13.64)
*rs*763857	75	32	0.748	0.384	44.00	53.13	0.58 (0.14-2.40)	0.80 (0.19-3.41)
*rs*1997547	16	11	0.086	0.875	12.50	45.45	0.40 (0.02-10.02)	2.60 (0.14-50.05)
*rs*2106234	61	32	0.150	0.837	32.79	50.00	0.40 (0.16-1.04)	0.45 (0.12-1.72)
*rs*648181	36	19	0.018	0.161	25.00	31.58	0.64 (0.18-2.35)	0.64 (0.15-2.84)
*rs*655988	39	31	0.228	0.853	30.77	32.26	0.30 (0.03-3.13)	0.29 (0.03-2.79)
*rs*371267	40	30	0.071	0.020	30.00	23.33	1.40 (0.46-4.30)	0.98 (0.26-3.75)
*rs*612592	68	32	0.748	0.419	33.82	25.00	1.68 (0.65-4.35)	N/A (N/A)
*rs*676982*	69	33	0.980	0.450	39.13	42.42	0.63 (0.25-1.56)	0.23 (0.06-0.87)
*rs*624590*	33	32	0.175	0.344	21.21	40.63	1.88 (0.31-11.64)	7.00 (1.26-38.99)
*rs*695077	50	30	0.777	0.543	34.00	20.00	0.00 (N/A)	0.00 (N/A)
*rs*11220409*	40	30	0.013	0.122	30.00	33.33	0.45 (0.13-1.58)	0.32 (0.10-1.08)
*rs*17656	30	20	0.524	0.717	30.00	15.00	2.68 (0.62-11.57)	N/A (N/A)
*rs*499205	74	33	0.863	0.770	33.79	33.33	0.99 (0.41-2.39)	0.65 (0.10-4.21)
*rs*638766*	59	32	0.529	0.344	28.81	40.63	0.40 (0.15-1.06)	0.13 (0.03-0.59)
*rs*667627	57	31	<0.0005	<0.0005	17.54	3.23	6.32 (0.76-52.52)	0.95 (0.30-3.00)
*rs*591163*	75	21	0.028	0.501	41.67	42.86	8.89 (1.86-42.44)	10.67 (2.07-54.85)
*rs*8177374	24	149	0.624	0.637	25.00	26.85	0.92 (0.34-2.59)	1.22 (0.13-11.12)
*rs*8177376	89	33	0.392	0.400	33.70	18.18	2.28 (0.85-6.15)	0.91 (0.08-10.52)
*rs*1786704	113	33	0.865	0.461	31.86	33.33	2.45 (0.48-12.68)	2.88 (0.59-13.99)
*rs*648710	40	26	<0.0005	<0.0005	2.50	3.85	0.92 (0.05-16.50)	1.77 (0.62-5.01)
*rs*586566*	77	33	0.729	0.400	35.06	15.15	3.12 (1.07-9.09)	1.44 (0.26-7.98)
*rs*240537*	78	31	0.206	0.616	43.59	22.60	2.72 (1.04-7.12)	1.68 (0.17-17.13)

*rs*7937122	106	33	0.438	0.930	52.83	48.45	1.31 (0.55-3.12)	1.35 (0.41-4.45)
*rs*11220473	60	29	0.895	0.170	48.57	62.07	0.60 (0.22-1.67)	1.11 (0.27-4.51)
*rs*582037	92	33	0.958	0.171	44.57	36.36	2.39 (0.75-7.63)	2.05 (0.66-6.41)
*rs*2230279	108	32	0.064	0.083	46.30	46.88	1.05 (0.47-2.32)	N/A (N/A)
*rs*752806*	51	31	<0.0005	0.437	7.84	54.84	0.13 (0.03-0.50)	4.03 (1.10-14.78)
*rs*7925175	54	30	0.433	0.794	40.74	53.33	0.92 (0.35-2.40)	1.08 (0.27-4.29)
*rs*1943528	57	32	0.321	0.065	38.60	28.13	1.53 (0.39-5.95)	0.94 (0.26-3.33)
*rs*9971527	70	33	0.577	0.632	31.43	42.42	0.50 (0.20-1.23)	0.25 (0.06-1.08)
*rs*612841	76	32	0.212	0.258	42.11	56.25	0.31 (0.08-1.22)	0.43 (0.11-1.78)
*rs*625496	77	32	0.600	0.614	45.45	53.13	0.74 (0.29-1.86)	1.00 (0.29-3.49)
*rs*6590224*	41	153	0.713	0.002	39.02	21.57	1.21 (0.33-4.47)	0.48 (0.14-1.67)
*rs*4935987	40	35	0.800	0.554	45.00	54.29	0.95 (0.26-3.48)	1.60 (0.40-6.36)
*rs*10893564*	52	32	0.005	0.591	9.62	21.88	0.38 (0.11-1.33)	1.07 (0.09-12.37)
*rs*7127398*	41	31	0.262	0.081	34.15	61.29	0.33 (0.12-0.93)	1.14 (0.19-6.89)
*rs*1946050	33	24	0.009	0.221	24.24	33.33	0.67 (0.20-2.26)	1.17 (0.28-4.87)
*rs*11220621	58	33	0.209	0.602	39.66	48.48	0.39 (0.09-1.63)	0.47 (0.11-1.97)
*rs*1106804	30	31	0.288	0.230	36.67	48.39	0.79 (0.27-2.28)	5.36 (0.55-51.71)
*rs*7110377	13	10	<0.0005	0.577	0.00	30.00	0.00 (N/A)	0.00 (N/A)
*rs*10750355	33	33	0.036	0.602	30.30	48.48	0.23 (0.05-1.10)	0.40 (0.09-1.83)
*rs*10893604	72	32	0.346	0.684	41.67	53.12	0.88 (0.28-2.78)	1.67 (0.49-2.71)
*rs*1628588	71	33	0.450	0.707	45.07	51.51	0.59 (0.18-1.88)	0.65 (0.19-2.25)
*rs*1793668*	62	29	<0.0005	0.139	14.52	20.69	0.17 (0.03-1.00)	0.19 (0.04-0.88)
*rs*2508557*	53	32	0.291	0.039	41.51	31.25	0.99 (0.33-2.93)	0.38 (0.12-1.17)
*rs*7941136*	51	32	0.674	0.540	29.41	50.00	0.35 (0.13-0.90)	0.12 (0.01-1.30)

*: SNPs chosen for validation genotyping

Table 5: Chromosome 11 Validation Genotyping for Interesting SNPs

SNP	# Cases	# Controls	HWE p-value Case	HWE p-value Control	% Het Case	% Het Control	OR Heterozygote (95% CI)	OR Homozygote (95% CI)
*rs*2587550	157	166	0.001	0.514	30.57	43.98	0.39 (0.18-0.84)	0.64 (0.30-1.36)
*rs*2734839	155	163	<0.0005	0.351	16.13	43.56	0.38 (0.21-0.68)	2.87 (1.62-5.08)
*rs*12361261	62	119	<0.0005	<0.0005	1.61	2.52	0.62 (0.06-6.14)	0.86 (0.31-2.40)
*rs*1509513	117	168	0.223	0.368	40.17	52.38	0.52 (0.31-0.88)	0.60 (0.29-1.22)
*rs*1713676	149	164	0.027	0.631	40.94	51.83	0.62 (0.36-1.07)	0.92 (0.50-1.69)
*rs*1176746	132	163	0.979	0.456	40.91	44.79	0.70 (0.43-1.14)	0.38 (0.18-0.84)
*rs*676982	166	168	0.084	0.036	33.73	33.33	1.00 (0.63-1.60)	0.89 (0.43-1.85)
*rs*624590	140	165	0.004	0.064	27.14	33.94	0.82 (0.36-1.87)	1.16 (0.54-2.5)
*rs*11220409	110	137	0.549	0.582	43.64	38.69	0.40 (0.15-1.05)	0.26 (0.10-0.68)
*rs*638766	160	168	0.009	0.048	28.75	33.93	0.76 (0.47-1.23)	0.78 (0.37-1.64)
*rs*591163	144	158	0.388	0.016	41.67	33.54	1.19 (0.58-2.47)	0.80 (0.40-1.63)
*rs*586566	177	175	0.128	0.500	33.33	32.57	1.09 (0.69-1.71)	1.57 (0.68-3.66)
*rs*240537	174	169	0.845	0.901	40.80	36.09	1.27 (0.81-1.98)	1.42 (0.59-3.39)
*rs*752806	152	172	0.004	0.718	38.16	48.26	0.89 (0.52-1.50)	1.82 (1.01-3.27)
*rs*6590224	140	135	0.423	0.711	34.29	25.93	0.55 (0.16-1.89)	0.34 (0.10-1.13)
*rs*10893564	134	169	<0.0005	0.722	14.93	28.40	0.46 (0.26-0.82)	2.21 (0.65-7.54)
*rs*7127398	39	31	0.215	0.081	33.33	61.29	0.33 (0.12-0.91)	1.19 (0.20-7.23)
*rs*1793668	164	165	0.002	0.815	35.37	40.00	0.40 (0.19-0.82)	0.40 (0.20-0.81)
*rs*2508557	150	173	0.686	0.203	46.67	45.09	0.85 (0.51-1.40)	0.56 (0.30-1.04)
*rs*7941136	139	159	0.630	0.343	31.65	32.08	0.93 (0.57-1.52)	0.43 (0.13-1.42)

CHAPTER 5

DISCUSSION

The genetic basis of susceptibility to SCC has yet to be determined. Through genome wide association studies, the whole genome can be scanned for genetic abnormalities within disease populations. Through these kinds of studies, allelic imbalance and loss of heterozygosity can be identified in cancers, leading to the elucidation of susceptibility loci and candidate genes.

Additionally, ROH is thought to be implicated for multiple diseases. ROH is characterized by the presence of consecutive homozygous markers, such as SNPs. ROH has been identified in a few cancers, such as breast and prostate cancer. The presence of ROH in cancer patients may indicate its influence on cancer predisposition at the genetic level.

The hypotheses for this study were to determine if ROH are common in individuals with skin cancer (SCC), and to identify potential SNPs that are associated with SCC risk. Normal DNAs were ascertained from cancer patients (cases) and non-cancer individuals (controls) and genotyped at 123 SNPs by Sequenom Technology. These SNPs were hand-picked at regions on chromosome 7 and chromosome 11 that have been previously identified as potential candidate regions for SCC susceptibility genes. Regions on human chromosome 7 mapped to the susceptibility locus *Skts5* on mouse chromosome 12. The regions of 11q23-11q24 on chromosome 11 have been previously implicated in SCC susceptibility based on preferential allelic imbalance studies.

Two rounds of Sequenom genotyping were conducted. Initially, 53 SNPs mapping to chromosome 7 and 70 SNPs mapping to chromosome 11 were genotyped. Frequencies of heterozygosity, HWE and genotype distribution, and odds OR for SCC risk were calculated for both the case and control populations. After initial genotyping experiments, ten SNPs from

chromosome 7 and 20 SNPs from chromosome 11 were chosen for further genotyping to validate the results by increasing the sample size. With a larger sample size, we looked to confirm our previous results and see the same trends in heterozygosity, variations in genotypic distributions, and odds ratio for SCC risk or protection as before.

5.1 Chromosome 7

Overall, the percentages of heterozygosity in cases and controls were similar and showed no significant differences across all 53 SNPs on chromosome 7. Additionally, no ROH were identified to be significant and unique to just the case population. The three SNPs *rs*2520458, *rs*6974011, *rs*12699991 were identified as candidate protective SNPs, and the *rs*3807917 was found to cause an increased risk of SCC.

After secondary genotyping, we found two regions of particular interest that were within and around the gene *Hdac9* (Figure 8). *Hdac9* encodes the enzyme histone deacetylase 9. Histones are important in transcriptional regulation, cell cycle progression, and developmental events. The acetylation and deacetylation of histones can alter chromosome structure, thus affecting a transcription factor's access to DNA.

The first region in *Hdac9* contains two significant SNPs (*rs*3807917 and *rs*2520458) and maps to a 6kb region on chromosome 7. We hypothesized that the cases would have a lower percentage of heterozygosity (and a higher proportion of homozygous alleles), and found that the case population experienced a 1.33 decrease in heterozygosity in this region (53.82% of controls and 40.49% of cases). SNP *rs*3807917 was calculated to have an OR of 2.3 (CI: 1.11-4.74), indicating that the homozygous genotype of TT causes an increased risk of SCC. The SNP *rs*2520458 was identified to be protective in the heterozygous state (GT), with an OR of 0.55

(CI: 0.34-0.89). At this SNP, the case population exhibited a 1.4 fold decrease in percent heterozygosity compared to controls. These two SNPs are located within *Hdac9* introns. However, according to one shorter *Hdac9* splice form (USCS Genome Browser), these two SNPs are located before the gene's 5' start site. If upstream of *Hdac9*, the significant SNPs found could alter gene expression through some sort of regulation. If they are found within non-coding introns, they could affect gene and protein expression by altering mRNA splice sites. In addition to these possible genetic implications, the SNPs could also affect a later functional event that we have not studied yet.

A second region of 22.3kb also maps to H*dac9* on chromosome 7. We identified two important SNPs here. The case population at *rs*6974011 had a genotype distribution that deviated from HWE at p=0.038. Additionally, the heterozygote (CA) and homozygote (AA) genotype both had OR < 1, indicating that both genotypes are implicated in SCC protection. This also indicates that the 'A' allele acts in a dominant manner and plays a role in resistance, as its presence in either the heterozygote and homozygote genotype results in a protective state of SCC. Also in this region was *rs*12699991. The case population here had a genotype distribution that deviated from HWE at p=0.001. This SNP is identified to be protective in the heterozygous state (AG) with an OR=0.57 (CI: 0.35-0.94). Also significant was a 1.4 fold decrease in the cases compared to the controls (51.47% in the cases and 35.80% in the controls).

This region of interest we identified (*rs*6974011 and *rs*12699991) was found to affect the occurrence of SCC. Having a heterozygous genotype at each of these SNPs seems to be an indicator of SCC outcome and a lower prevalence rate of SCC. The identification of a number of SNPs that are associated with protection for SCC can provide additional information to the exact role of *Hdac9* in this disease. These polymorphisms in the intronic non-coding region of the

gene could affect mRNA splicing, gene expression, and protein production in some way that confers a greater resistance to the disease. Other studies in Dr. Toland's lab have seen an increase in Hdac9 protein levels in skin cancer resistant *Mus Spretus*, indicating a possible role of Hdac9 in regulating and silencing oncogenes that contribute to SCC resistance (Fleming, J.L. personal communication).

5.2 Chromosome 11

Chromosome 11 did not exhibit significant differences in percent heterozygosity between the case and control population. A number of individual SNPs exhibited noticeably different heterozygosity percentages. No ROH was identified to be significant and unique in the case population when compared to controls. The case population deviated from HWE ($p<0.01$) at 8/20 SNPs (40.00%) that were genotyped for validation. Control populations deviated from HWE ($p<0.01$) at 1/20 SNPs (5.00%) that were genotyped for validation, suggesting an increase in deviation from HWE in the cases compared to controls. A number of SNPs were implicated in increased SCC risk or resistance, as mentioned earlier.

Two of the significant SNPs, *rs*2587550 and *rs*2734839, were located in or around the gene *DRD2*, the dopamine receptor D2 (13.5kb region) (Figure 9). This gene encodes the D2 subtype of the receptor for the neurotransmitter dopamine. Case populations for both SNPs deviated from normal HWE ($p<0.01$). Both were also found to be protective of SCC in the heterozygous state; heterozygotes at these SNPs seem to have a lower risk of SCC development. *rs*2587550 occurs upstream of *DRD2,* potentially affecting functional characteristics we have not studied, such as gene regulation by enhancers and suppressors. In addition to being protective in the heterozygous state, SNP *rs*2734839 was significant in that it indicated an increased risk of

SCC in the homozygous state (GG) (OR: 2.87, CI: 1.62-5.08). This SNP exhibited a noticeable change in percent heterozygosity; the case population had a 2.7 fold decrease in percent heterozygosity (43.56% controls and 16.13% cases).

Our findings of susceptibility and risk SNPs at *DRD2* require further investigations and functional studies to determine whether or not it is a susceptibility gene to SCC. With its significant OR and the large difference in homozygosity and heterozygosity frequencies between the cases and controls, *rs*2734839 seems to affect SCC risk. Individuals with a genotype of GG at this SNP could be more susceptible to SCC than individuals with a heterozygous genotype of GA. This suggests that the 'G' allele acts in a recessive manner, since two copies of the allele are associated with and predictive of the SCC phenotype and one copy of the allele was not. Thus, individuals with homozygous GG are at a higher risk of developing SCC than those with a heterozygous genotype. In previous studies, other polymorphisms in *DRD2* have been associated with colorectal cancer and non-small cell lung cancer[21,22]. Its role in skin cancer is unknown, and additional research is needed to find its genetic influence in SCC.

Another region of interest that included SNPs *rs*752806, *rs*10893564, *rs*7127398, and *rs*1793668, was centered around the gene *KIRREL*3 on 11q24 (Figure 9). *KIRREL3*, kin of IRRE like 3 encodes a protein of the nephrin-like protein family that is expressed in the fetal and adult brain and podycytes of the kidney. Case populations at three SNPs (*rs*752806, *rs*10893564, and *rs*1793668) deviated from HWE ($p<0.01$) while the controls stayed within normal expected values and HWE. SNP *rs*752806 is located upstream of the *KIRREL3* start site. In addition to the genotype distributions deviating from HWE, the homozygous state (TT) of this SNP is associated with an increased SCC risk (OR: 1.82, CI: 1.01-3.27). Its upstream location indicates that it may affect gene regulation and downstream expression.

The other three SNPs in this region are all located within the 166kb of the gene *KIRREL3*. *rs*10893564, *rs*7127398, *rs*1793668 are all identified as protective SNPs to SCC. The case population exhibited a 1.8 fold decrease in heterozygosity at *rs*7127398 compared to controls (61.29% in controls and 33.33% in cases). Also, *rs*1793668 shows interesting characteristics: it is protective for SCC in both the heterozygous (CT) and the homozygous (TT) state. This suggests that the 'T' allele acts in a dominant fashion, resulting in a protective state or phenotype whenever it is present in the genotype. The presence of the protective SNPs suggests that *KIRREL3* is a potential candidate resistance or tumor suppressor gene that promotes a protective phenotype to SCC. The distribution of the case population at these SNPs were $p<0.01$ (case population genotype distribution was non-significant with $p>0.01$), indicating that some characteristic of these SNPs is pushing the population out of normal HWE, whether it is a specific allele at the SNP or its functional targets. These SNPs are located in introns, and could alter the mRNA transcript and splicing activity in a way that contributes to SCC resistance. *KIRREL3* and its relationship to cancer have not been thoroughly studied, and further investigation of its effect on cell growth could provide more information on its exact functions.

5.3 Conclusions and Future Work

We were not able to prove the first hypothesis of this study. ROH for the regions included in this study did not appear to be more common in SCC cases than controls. Even though a handful of SNPs exhibited noticeably different rates of heterozygosity and homozygosity between cases and controls, homozygosity overall does not seem to have a significant effect on SCC prevalence and susceptibility. Therefore, homozygosity and ROH on chromosome 7 and 11 do not seem to play a role in SCC predisposition. Because this was not a

genome wide association study, we cannot conclude that ROH is not prevalent at other loci SCC cancer patients. It is possible that if thousands of SNPs across the whole genome were scanned, ROH important in SCC susceptibility could be identified on other chromosomes.

Our second hypothesis was to determine SNPs associated with SCC risk and protection. We identified a number possible candidate genes housing SNPs associated with increased SCC risk and resistance. *Hdac9* on chromosome 7 contains three SNPs (*rs*2520458, *rs*6974011, *rs*12699991) associated with protection and one SNP (*rs*3807917) associated with increased SCC risk. *DRD2* on chromosome 11 appears to also be protective in heterozygous states and cause increased risk in homozygous states at a few SNPs (*rs*2587550 and *rs*2734839). *KIRREL3* also houses three SNPs associated with protection in the heterozygous state (*rs*10893564, *rs*7127398, *rs*1793668). These three genes seem to be potential candidate SCC susceptibility or tumor suppressor genes. SNPs from introns and non-coding regions have the potential to alter gene expression. These areas are important in gene regulation; the consequences of SNPs include the alteration of splice site sequences that are used by spliceosomes, or a change in the ability of transcription factors to bind and regulate transcription.

These SNPs and genes warrant additional study on their potential role in SCC. Currently, there is little literature on the relationship between *DRD2*, *KIRREL3* and skin cancer. Functional studies can be done to determine the effect these genes have on SCC development. This includes cell culture studies, in which proliferation, apoptosis, and protein production can be measured. Findings from future studies may yield insight on the importance of these two genes in SCC.

Because the SNPs in this study were chosen from previous experiments, there is a possibility that other significant SNPs on chromosome 7 and 11 were not included in this study. Additionally, other SNPs associated with SCC could be located on chromosomes that were not

studied here. By conducting two rounds of genotyping in this study, it is possible that non-significant SNPs from the initial round of genotyping round could in reality, be significant. A complete genomic study could potentially be used to identify not only other ROH, but also additional SNPs that may be important in SCC susceptibility.

5.4 Significance

Runs of homozygosity of normal DNA in cancer patients is a relatively newly described phenomenon. Significant increases in homozygosity and ROH throughout the genome in normal DNA from SCC patients can indicate regions that may harbor susceptibility genes in skin cancer. Additionally, by studying the allele and genotype distribution across SCC patients and controls, each SNP can be analyzed for ORs. SNPs with differing genotype distributions between the cases and controls can be linked to SCC as either protective or susceptibility SNP genotypes. The genes that these SNPs reside in can then be identified as candidate genes that affect cancer predisposition.

Genetic studies of cancer are often conducted not only to understand the biology of the disease but also the development of prevention and treatment options. In order to identify future novel and effective target therapies, it is important to understand SCC at a genetic and etiologic level. The ability to identify tumor suppressor genes and oncogenes that are affected during tumor initiation and progression could facilitate the identification of novel molecular targets for therapeutic intervention and diagnostic biomarkers. Identified biomarkers could be applied and further used to determine both the predisposition to skin cancer and to predict the outcome of SCC in humans. Results from this study can help determine the genetic mechanism of the development and progression of SCC.

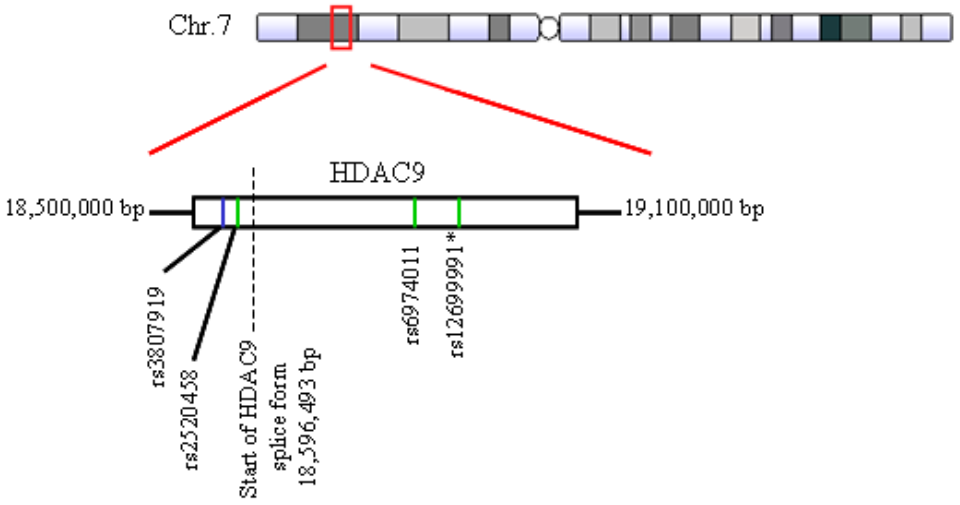

Figure 8: Significant SNPs on Chromosome 7. SNPs located within the introns of the gene HDAC9. SNPs *rs*3807919 and *rs*2520458 are located upstream of the gene based on a second HDAC9 splice form. Blue: SNPs with OR>1 indicating an increased risk of SCC. Green: SNPs with OR<1 indicates protection. Purple: SNPs with a genotype for protection and a genotype for increased risk of SCC. A * indicates a p<0.01 in the case population (deviation from HWE).

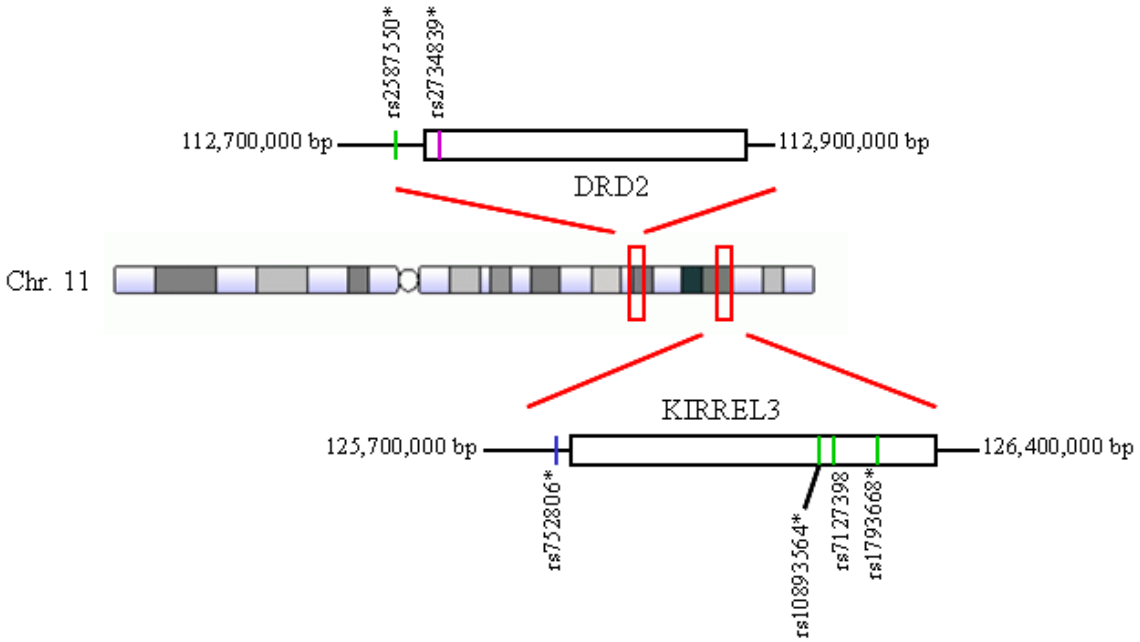

Figure 9: Significant SNPs on Chromosome 11. SNPs located at genes *DRD2* and *KIRREL3* on chromosome 11. Blue: SNPs with OR>1 indicating an increased risk of SCC. Green: SNPs with OR<1 indicates protection. Purple: SNPs with a genotype for protection and a genotype for increased risk of SCC. A * indicates a p<0.01 in the case population (deviation from HWE).

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SUPPLEMENTAL

Table 1: Sequenom Primers for Initial Genotyping

SNP	Primer	Sequence
CHROMOSOME 7		
*rs*12699991	Forward	CATAAATCGGATAAGGCCAT
	Reverse	TACAGGGTAGAAGGCGAAAG
	Extension	CTTCTTGCAGCCTTTGG
*rs*10486048	Forward	TGAACGTACGTTCTCTTCCC
	Reverse	CCACATTGAAAAAGGGATG
	Extension	CGTTCTCTTCCCATACTC
*rs*6968777	Forward	ATAGACTTTGCCAGAGGCCA
	Reverse	CCACATGTATGAGTTTCCTG
	Extension	GCCAGAGGCCAAATATAA
*rs*2520456	Forward	GGAGCAGAGGACCTACCAG
	Reverse	TGCAGCTGCAGCTATGGAC
	Extension	caCTACCAGGTCCCCGACT
*rs*2239926	Forward	TTGTGCTCACTGCTTAAGGG
	Reverse	GGAATCAGAGTTAGAAAAGTC
	Extension	ggTAAGGGACATCCTAGGG
*rs*801759	Forward	AATTGGAACTAGGCTGGGCG
	Reverse	TGACCTCAAGTGATCTGCCC
	Extension	CAGCGGCTCATGCCTATAAT
*rs*2520458	Forward	TCTCCAAGTTACGGATGAGG
	Reverse	GACTCTTTCATGTGTGTAAC
	Extension	gggCAGAGGCCTAGGGAAAT
*rs*7808451	Forward	GAAGCAAATAATGGCATCAG
	Reverse	TTCATATCCCTTTGATGCGG
	Extension	AGTGCTATAATGCACAAGAAA
*rs*12540224	Forward	TCATCCACTGGATACCTA
	Reverse	GGGTTACTTTTATAATAAAGC
	Extension	ccaCACTGGATACCTATGTGCT
*rs*2704284	Forward	GGTGTTGGAGGCATGAATTG
	Reverse	CTGCCCCTGGAGTTTTTAAC
	Extension	ggTGGAGGCTGAATGTGAACCA
*rs*2526633	Forward	TGTTGGAATGATTTCAGTGC
	Reverse	CGATCTTTATTCTTTTGTTGC
	Extension	TGGAATGATTTCAGTGCTCTTTA
*rs*2073973	Forward	TCAGCAATTTGCACTCTGCC
	Reverse	CAATTGAAGTCACCTGTTTG
	Extension	cccATTTGCACTCTGCCATGAATC
*rs*10486329	Forward	TAATGAAGGGTAGTTATC
	Reverse	CCTCTCTTGACACATATGAA
	Extension	taagGAAGGGTAGTTATCTCCTAA
*rs*1034805	Forward	GGCAGCAAGGTACAAAACAC
	Reverse	TTCAACTGTGTGGAGTTAC
	Extension	catggACAAAACACACTTTGCTTTA
*rs*10237366	Forward	TACCTAGCGAAGAAAGAAGG
	Reverse	CTCTCTTCTGAGTGAGATTC
	Extension	ggaTAGCGAAGAAAGAAGGAACTCT
*rs*6461065	Forward	CTGGAAAGATACTTGCAGAT
	Reverse	AGCTACCCACAGTCAGTATC
	Extension	gcTGCTACAAAGATTAATCAACAGGT

*rs*2040623	Forward	TCAGTAACTGCTGTCTCTGG
	Reverse	CTAATGGAAAAAGCATAAATG
	Extension	cctTCTCTGGCCACTCAGGAATACATC
*rs*1554774	Forward	TACAAAAGGTACATATTTTC
	Reverse	GCCAGAAGAGGCATACATTG
	Extension	ttcACATATTTTCAAAAGTACTACTTG
*rs*8177374	Forward	GCCGAGGGCTGCACCATCC
	Reverse	GTACATGAATCGGAGCTCAG
	Extension	CACCATCCCCCTGCTGT
*rs*10225248	Forward	GCTTTATAGATTTAGGTGC
	Reverse	AGGATCAATAGAGCAAAAGG
	Extension	GATTTAGGTGCTCCGAT
*rs*11764843	Forward	CCAAAGAGAGCTGTTCGATG
	Reverse	ACTTTCAGTTAATGCTGAG
	Extension	TGCCCTGCTGTTTTTTAT
*rs*1405618	Forward	GATGGTCCTGGGAATTTATC
	Reverse	CACAGTTTAACAACGATAGC
	Extension	ATGACCGGGGAAAGAGTG
*rs*17140399	Forward	GGATCTAGCACTTGGCTAAC
	Reverse	TTATCTCACTCTGGGCTGAC
	Extension	acGGCTAACTGGCTGTAAT
*rs*3213615	Forward	TGCCCCCAGCATAAGCAAAC
	Reverse	TGTCATGAATCAACTGATGC
	Extension	ctCAAACAATGCACACACAT
*rs*3807917	Forward	GGAAACTGCAAATGACTTGTG
	Reverse	CTCCTGCCTACCTTTCTGAC
	Extension	AAATGACTTGTGATTGGACT
*rs*2214867	Forward	CTGTTCTTTTCTGATAATGC
	Reverse	TGCAAACAGTATTCCACAGG
	Extension	TGCAATTCTGTTTGTCTTACT
*rs*7796078	Forward	GAAAGAAAAGATGCTGGTGG
	Reverse	TCTGCACAAATGATCTTGCC
	Extension	ggAGATGCTGGTGGAAATCAT
*rs*11543651	Forward	CTTCAAAATATCCTGAGGG
	Reverse	GCTGTGAAGGTAGAGAGAAT
	Extension	gtcATATCCTGAGGGTTTTTTG
*rs*6461387	Forward	TCCAAGTGACAACCCATAG
	Reverse	CCATGGTGACATGATAGAG
	Extension	cGTGACAACCCATAGTCTAACTA
*rs*726116	Forward	GGCTAATTTTCAGAGAAAGTG
	Reverse	CAATAAATAAGAAACATTTG
	Extension	CAGAGAAAGTGGTAATTCAATGC
*rs*6947529	Forward	CCTAAAGGAAACTATTTACTC
	Reverse	CTTCATCATAAAGCTAACC
	Extension	ccTACTCACATTTAACACCAAACT
*rs*2853552	Forward	TTCTGGGAACCTCACAGATG
	Reverse	AGGCATTAGGGATACTTGTG
	Extension	GGAACCTCACAGATGTTTCTATTA
*rs*3801986	Forward	ACATTAAGTAGGGGCCTTTC
	Reverse	GACATACTGTAAAGCACAAC
	Extension	gTTAAGTAGGGGCCTTTCTTTAGAG
*rs*17350355	Forward	TCAACCCTTTTTCAGGAACG
	Reverse	AACAGCATGGAATAAAGACC

	Extension	cccttAACGACTTTGTCTAGTCATAT
*rs*2237298	Forward	ACCTATAATGCTTGTCTACC
	Reverse	AATACTTGTCAGCAAGTTA
	Extension	agAATGCTTGTCTACCTCATTGTGTA
*rs*6974011	Forward	TAGCCCTATCATACATGTGG
	Reverse	CCTGAGGAATGTGCATAAGG
	Extension	ggggCTAGATTCTTAGGAATCTGATG
*rs*2158768	Forward	AAAAATGCCAGCCGAGTGTG
	Reverse	GTACACATGCCACTGAAACC
	Extension	TGGCTACCCAGGTGAAG
*rs*12700003	Forward	TGTGGAAGACCCTTCCACTG
	Reverse	GTGGTGCACGTTGAGACAG
	Extension	GCTATGTCCACCTCAGAA
*rs*7811989	Forward	TCTGAACACCTGCATAGGAG
	Reverse	GAGATAACAGACAAGTCCAC
	Extension	TAGGAGTTGACTGTAGGGA
*rs*7783171	Forward	GAAAATGATGACCAAAACAGG
	Reverse	GGGAAAATATCATCTCTCACC
	Extension	ACCAAAACAGGATTTTGCTT
*rs*2695027	Forward	CAACTGGTGCATTTGGTATC
	Reverse	CCATCTTACACCAAGTCAAG
	Extension	TTCAAAAGGTTTAGCAGATT
*rs*2158041	Forward	CTTGATGCCAACCATTACAC
	Reverse	GGGGTGTGTATATTTCAAC
	Extension	GCCAACCATTACACAATTTCC
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CHROMOSOME 11		
*rs*6589400	Forward	GTGCAATTCCTTTTGCTGGG
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	Extension	ACTGCCTTCCCCAAA
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	Reverse	CACTCATTTCAGACACACAC
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	Extension	gatgCCCACTCATACCGCT
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	Extension	CCTTCAGTCTTCCTAACTTC
*rs*7110377	Forward	GGCCATCTTGTCTTCACTTG
	Reverse	CAATCTGTCTACCTCTCAGC
	Extension	cgttTCTTCACTTGTTCCCC
*rs*1946050	Forward	GCAGATAGAGCAATGGGTTC
	Reverse	ATCACATAACTGGCCCCTTG
	Extension	CTGGCTTTTGCCTGCATTAA
*rs*2734839	Forward	CGCAACTAGATGTTTAAGGC
	Reverse	ATACTCTGGTATCTGCTGTG
	Extension	GCCTGGTCATCTTAGATATA
*rs*7937122	Forward	ACACTGGTGCAATGCCAAAC
	Reverse	TTGTTCCTCTGCAGGTGACT

	Extension	caCGTGCAAGGAATGATTTAG
*rs*1713676	Forward	AGAAGTCTGAGGACTCCAAG
	Reverse	ACCTGCTCAATGTCGGTGTC
	Extension	ggtggGGTAGCAGAAACCAGC
*rs*638766	Forward	TCAGGTTTCTCTGGTTAAGG
	Reverse	CAGTTACTTGCCATGCTCTG
	Extension	TTGCCTGCAACTACATCTGTTA
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	Extension	ccagCCTTGGCTTACAGATTGT
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	Reverse	TCCCACATTTAATGCGTTCC
	Extension	tCTAGAGAAGAGCAGATGAGAA
*rs*582037	Forward	TGGAGTCAGACCCTCCAGC
	Reverse	TGGAGCAGCCCTTCCCTGT
	Extension	cccttAGACCCTCCAGCAGCACC
*rs*624590	Forward	ACCCACCCCCTGCAAATTTA
	Reverse	CTTTTAGGGTGGAATTTCTC
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	Reverse	ATATGCCCATTACCCAGGTC
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	Reverse	GTGACCAGTTGGTGTTTAAG
	Extension	ctccCAGTGCCCTGAGAAATATCCACAC
*rs*591163	Forward	CTGTGGTCCAAGGCCATTTT
	Reverse	TTAAGAAGAGCTGTGTGCCC
	Extension	cctaCAAGGCCATTTTTGCTGGCTGTAC
*rs*240537	Forward	CCAGGAACACACTGGAATCT
	Reverse	GTGAGTGGCTTCACCAAACC
	Extension	gattGAATCTGAGTCTAATTTCTCTGAG
*rs*625496	Forward	CCTGCTTTCACACCGTCAG
	Reverse	CCTGTGCAGTTTTGATGCTC
	Extension	CACCGTCAGTGGGAT
*rs*6276	Forward	TTCCTGCTCACGGTTCGCAA
	Reverse	ATCCTCCACTGCTGACTCTG
	Extension	AAGGGTGAGGCTGGC
*rs*586566	Forward	TAACCCCGCTTTCATACGTC
	Reverse	GTTTCACGGTGTGATACTGG
	Extension	GAAGAGGGTGGGCTGG
*rs*612841	Forward	TTCTTGGCACACGTTCAAGG
	Reverse	CAGACTCTCTTAGTACAGCG
	Extension	ACGTTCAAGGGCCCACA
*rs*11214677	Forward	CAAACTCAGCTCATACAGAC
	Reverse	ATCCTCAATACTGCACTCCC
	Extension	AGACTCTGGGATAGGAA
*rs*1628588	Forward	GTATGGCAGTTGAAGGACAC
	Reverse	TGAGCGAGTAGGCAATGAAC
	Extension	ttGACACACGACTTGAGG
*rs*4471464	Forward	GGTCTAATGAGACAAAAGGC
	Reverse	TTCTATGGCTGTCTTAAGAG
	Extension	GGTCTGCTTGGAAAGAGA
*rs*499205	Forward	ACCTGAATTTGGTCACCTTC
	Reverse	ATGCTTGGCCATCACCAAAC
	Extension	ggACCTTCATCCTGTTGTCC
*rs*1786704	Forward	CCTAGAAAATCTTGCTTGTG
	Reverse	GGTGTTCCTAGGTTTTTGC
	Extension	gaAAAAAAGTTCAGAGGCTC
*rs*763857	Forward	CCCTGGCATTGTCTTCATCA
	Reverse	ATCCCTTCAACCTCTCACCT
	Extension	aAAGAATGGGGTTAGGGATC
*rs*10893604	Forward	GAAAGCCTGCATGTTTATTG
	Reverse	GCAGAAGGAAAGGGAATTGG

	Extension	TGTTTATTGATAACAACCTGA
*rs*11214716	Forward	GTCTACATACATGTAATGG
	Reverse	CCTTCAGAAATAAAGATGAG
	Extension	GATTATTATTGTTGAAGTGAGAC
*rs*11220621	Forward	CAGATGACATTTCTTTGTTAG
	Reverse	CCCACCTCCTGCTAACATTT
	Extension	TTCTTTGTTAGAAGTATAGGATTTT

Table 2: Sequenom Primers for Validation Genotyping

SNP	**Primer**	**Sequence**
*rs*12699991	Forward	CATAAATCGGATAAGGCCAT
	Reverse	TACAGGGTAGAAGGCGAAAG
	Extension	CTTCTTGCAGCCTTTGG
*rs*2508557	Forward	AAGAGTCAGTGCGAGTGTGG
	Reverse	ACAAGGCTAGTGAAGTGTGC
	Extension	GAGTGTGGAGTGCAGAC
*rs*11220409	Forward	CTAACTCACAGTGATAGGAAC
	Reverse	GCATGGAAGGCAAAAAATAG
	Extension	TGATAGGAACTTGCGCCA
*rs*3213615	Forward	TGCCCCCAGCATAAGCAAAC
	Reverse	TGTCATGAATCAACTGATGC
	Extension	cCAAACAATGCACACACAT
*rs*801759	Forward	AATTGGAACTAGGCTGGGCG
	Reverse	TGACCTCAAGTGATCTGCCC
	Extension	CAGCGGCTCATGCCTATAAT
*rs*6590224	Forward	GGAAGGATGACGGTTTTGTG
	Reverse	CAAGATGCTCTCACAGATGG
	Extension	ggTGACGGTTTTGTGCCTTAT
*rs*2695029	Forward	TCCAGCATGTTAAGATGGC
	Reverse	ACTACACAGTCCCTTACATC
	Extension	GGCAAAGAGAAATGAGTAAAA
*rs*586566	Forward	TAACCCCGCTTTCATACGTC
	Reverse	GTTTCACGGTGTGATACTGG
	Extension	gggGTGAAGAGGGTGGGCTGG
*rs*240537	Forward	CCAGGAACACACTGGAATCT
	Reverse	GTGAGTGGCTTCACCAAACC
	Extension	AATCTGAGTCTAATTTCTCTGAG
*rs*1509513	Forward	AGAAGAGGATGGAACTCTGG
	Reverse	CATCTCAATCTTCTTTGTGGC
	Extension	gagGGAACTCTGGAGAGCAATAA
*rs*2158041	Forward	CTTGATGCCAACCATTACAC
	Reverse	GGGGTGTGTATATTTCAAC
	Extension	gGATGCCAACCATTACACAATTTCC
*rs*1176746	Forward	ATCTGGGCCCCCGATATCAT
	Reverse	TACCAAGCAGTCTAAACCCC
	Extension	gTCATCAATGAGTTGTAAGTGTGCC
*rs*2520458	Forward	GACTCTTTCATGTGTGTAAC
	Reverse	TCTCCAAGTTACGGATGAGG
	Extension	agtTGTGTAACATTGAAGATGTTATT
*rs*752806	Forward	CCACAGGGGAGAGGAGTCA
	Reverse	GCAGTTGATAGCTCTGATTG

	Extension	gtGGAGAGGAGTCAGCCTGTAGGTTT
*rs*676982	Forward	TTCTTCTGCTAGCCACAACC
	Reverse	TGACTTGTGTGAGAATGTGC
	Extension	TAGCCACAACCATCTCT
*rs*12361261	Forward	TTTTGTTGCCTCCATCCAGC
	Reverse	CTTGGTCTGTTATAGCTGCC
	Extension	CCAGCCCCAAAGGGCTA
*rs*3807917	Forward	CTCCTGCCTACCTTTCTGAC
	Reverse	GGAAACTGCAAATGACTTGTG
	Extension	cTTCCACCTGTGCCTTGTA
*rs*1793668	Forward	TCACAGGGACTCAGAGTCAT
	Reverse	TTCTGGCACCCATGTCTACC
	Extension	CAGAGTCATGAGGCCCTTC
*rs*2587550	Forward	ATTTTAAAAGCCCCTTGCCC
	Reverse	AGCTGGAGTGTGTCCTAGAG
	Extension	TAACGTGCAGGCTCGGTTTA
*rs*2237298	Forward	ACCTATAATGCTTGTCTACC
	Reverse	AATACTTGTCAGCAAGTTA
	Extension	cCTTGTCTACCTCATTGTGTA
*rs*10893564	Forward	AATGTGCCAGCACAGCTTTC
	Reverse	CAGGGATATACTGCAAACG
	Extension	ggggTTTGCTTTGAGCAGTGT
*rs*2734839	Forward	ATGTTTAAGGCTGCCTGGTC
	Reverse	GCTCACAACCGTGTTTTTAA
	Extension	CTGCCTGGTCATCTTAGATATA
*rs*624590	Forward	ACCCACCCCCTGCAAATTTA
	Reverse	GTAATAATTCTTTTAGGGTGG
	Extension	ccCCCCCTGCAAATTTACCAGTA
*rs*591163	Forward	CTGTGGTCCAAGGCCATTTT
	Reverse	TTAAGAAGAGCTGTGTGCCC
	Extension	ccccGCCATTTTTGCTGGCTGTAC
*rs*6974011	Forward	TAGCCCTATCATACATGTG
	Reverse	CCTGAGGAATGTGCATAAGG
	Extension	TTCTAGATTCTTAGGAATCTGATG
*rs*638766	Forward	TCAGGTTTCTCTGGTTAAGG
	Reverse	CAGTTACTTGCCATGCTCTG
	Extension	gcttgGCCTGCAACTACATCTGTTA
*rs*7941136	Forward	TCGGTTTGTTCAAAGGCCTG
	Reverse	ACAAACTTTCCAGGAACAGG
	Extension	gggaATACTTAAAGACATGTGGAAT
*rs*1713676	Forward	ACCTGCTCAATGTCGGTGTC
	Reverse	AGAAGTCTGAGGACTCCAAG
	Extension	gctaAATGTCGGTGTCCAGGACCGCA
*rs*801763	Forward	TATGTGGTCGCTTCTGACTG
	Reverse	AGTACTGAGTTACATGCTAC
	Extension	cctgaCTTCCTTTACTCAGCATGGTTT
*rs*7127398	Forward	AGACCATGTGATACTAGCCC
	Reverse	TTGGTTGCCATCCCATACAG
	Extension	ACTAGCCCCAAAGGGAG

Table 3: Primers used to sequence Sequenom-designed primers

SNP of Sequenom Primers	Primer	Sequence	T_m (°C)
*rs*752806	Forward	ACA AAG CCA CAG GAA GAA CCC AAG	59.7
	Reverse	TTC TGG ACC CTT CCT CTC AGC AAT	59.9
*rs*638766	Forward	TGT TCA CTG TCT TGG GCT CTG GAT	60.2
	Reverse	ACG AGT CTG TAG TAG CAC AAG CCT	59.5
*rs*1713676	Forward	GCA GTA GAA ATG CGG TTG GAC AGT	59.6
	Reverse	ACA CAG GTT CTG GAG GAG GTC AAT	59.8
*rs*2587550	Forward	AAG GAA GGG CAC AGA GAG AAA CCA	60.5
	Reverse	ATG GAG GAC ACA AAG CTG CTA GCT	60.4
*rs*2734839	Forward	ATG GAA AGA CTT GGG AGCA GTC CA	60.3
	Reverse	AGA ACG AGT GCA TCA TTG CCA ACC	60.1
*rs*12699991	Forward	AGG CTG GGA TCA AGT CCA AGA TCA	59.9
	Reverse	TCG CTG CTT AAA GGC CCT ACC ATT	60.7
*rs*10893564	Forward	AGA GTG GAG CCA TCT GCT CAT CTT	59.9
	Reverse	TTG TGG GTA AGC TGA CTT GGG ACA	60.2
*rs*801763	Forward	GGT TGT GCA ACC ATC ACC ACA ATC	59.0
	Reverse	TTG CTG GCT CGT AAG TAC TTG CCA	60.6
*rs*7941136	Forward	GCT GTC AGC CCA ATG TCT ATC ATG TG	57.3
	Reverse	ATC TGT CTG GGC TCT TCC TTT CCA	60.4
*rs*6974011	Forward	TGT CCA GTG CTT CCC TGC ATA ACT	60.4
	Reverse	CAA CAA ATC CTG GAA AGA GGT GAG	55.9
*rs*3213615	Forward	TGC CCA CCA CAC ATC ATT GGA TCA	61.0
	Reverse	CTT CTC CAT TAG ATA GGG CCT CTG	56.2
*rs*12361261	Forward	ATA TGT TTG AGG GCA GGA CGA CCA	60.2
	Reverse	AAC AGC ACT GAG CAG GAC TCT GAA	60.2
*rs*2520458	Forward	CTG TTA AGG GCT GTG CTA CAT GCT	59.3
	Reverse	TTT GAA GAG CCT GGT GCT GTG ACT	60.7
*rs*624590	Forward	AGT GGG AAA GAA GCA AGG CTT GTG	59.9
	Reverse	GGG ATG GGA GAG GCT GTT	56.9

Table 4: Initial Chr. 7 Allele and Genotype Frequencies

SNP	Cases - Alleles		Controls – Alleles		Cases - Genotypes			Controls - Genotypes		
	p	q	p	q	pp	pq	qq	pp	pq	qq
*rs*10225248	0.625	0.375	0.556	0.444	0.417	0.417	0.167	0.305	0.503	0.192
*rs*2214867	0.563	0.438	0.454	0.546	0.375	0.375	0.250	0.223	0.462	0.315
*rs*6461065	0.799	0.201	0.785	0.215	0.644	0.310	0.046	0.601	0.367	0.032
*rs*10486048	0.124	0.876	0.110	0.890	0.012	0.224	0.765	0.013	0.194	0.794
*rs*2237298	0.357	0.643	0.241	0.759	0.095	0.524	0.381	0.103	0.276	0.621
*rs*2158041	0.326	0.674	0.160	0.840	0.070	0.512	0.419	0.034	0.252	0.714
*rs*7811989	0.321	0.679	0.227	0.773	0.048	0.548	0.408	0.073	0.307	0.620
*rs*2040623	0.818	0.182	0.753	0.247	0.694	0.247	0.059	0.600	0.306	0.094
*rs*801759	0.101	0.899	0.048	0.952	0.000	0.203	0.797	0.000	0.096	0.904
*rs*11543651	0.222	0.778	0.268	0.732	0.111	0.222	0.667	0.114	0.309	0.577
*rs*801763	0.875	0.125	0.826	0.174	0.750	0.250	0.000	0.686	0.279	0.035
*rs*2520456	0.241	0.759	0.292	0.708	0.069	0.345	0.586	0.071	0.442	0.487
*rs*3807917	0.523	0.477	0.642	0.358	0.409	0.227	0.364	0.383	0.518	0.099
*rs*2520458	0.682	0.318	0.657	0.343	0.506	0.353	0.141	0.396	0.522	0.082
*rs*2073973	0.482	0.518	0.443	0.557	0.259	0.447	0.294	0.195	0.497	0.308
*rs*2695029	0.372	0.628	0.486	0.514	0.077	0.590	0.333	0.243	0.486	0.270
*rs*2695027	0.705	0.295	0.599	0.401	0.513	0.385	0.103	0.370	0.459	0.171
*rs*3213615	0.538	0.462	0.693	0.307	0.346	0.385	0.269	0.477	0.431	0.092
*rs*2239926	0.259	0.741	0.256	0.744	0.057	0.402	0.540	0.057	0.399	0.544
*rs*2704284	0.547	0.453	0.497	0.503	0.279	0.535	0.186	0.219	0.555	0.226
*rs*1405618	0.250	0.750	0.299	0.701	0.083	0.333	0.583	0.078	0.442	0.481
*rs*1554774	0.707	0.293	0.739	0.261	0.500	0.415	0.085	0.562	0.353	0.085
*rs*3801986	0.476	0.524	0.448	0.552	0.190	0.571	0.238	0.185	0.526	0.289
*rs*7796078	0.727	0.273	0.681	0.319	0.591	0.273	0.136	0.484	0.394	0.123
*rs*212664	0.268	0.732	0.341	0.659	0.122	0.293	0.585	0.145	0.393	0.462
*rs*17349342	0.452	0.548	0.388	0.612	0.214	0.476	0.310	0.133	0.510	0.357
*rs*7783171	0.802	0.198	0.805	0.195	0.651	0.302	0.047	0.638	0.336	0.027
*rs*12540224	0.465	0.535	0.433	0.567	0.200	0.529	0.271	0.179	0.506	0.314
*rs*6974011	0.533	0.467	0.316	0.684	0.333	0.400	0.267	0.096	0.440	0.464
*rs*12699991	0.606	0.294	0.599	0.401	0.435	0.341	0.224	0.353	0.494	0.154
*rs*2520362	0.488	0.512	0.517	0.483	0.233	0.512	0.256	0.227	0.580	0.193
*rs*2073964	0.369	0.631	0.379	0.621	0.143	0.452	0.405	0.144	0.471	0.386
*rs*6461387	0.375	0.625	0.43	0.57	0.200	0.350	0.450	0.158	0.544	0.298
*rs*12700003	0.581	0.419	0.559	0.441	0.326	0.512	0.163	0.314	0.490	0.196
*rs*10237366	0.376	0.624	0.413	0.587	0.129	0.494	0.376	0.155	0.516	0.329
*rs*17350355	0.368	0.632	0.403	0.597	0.211	0.316	0.474	0.161	0.484	0.355
*rs*11764843	0.619	0.381	0.581	0.419	0.476	0.286	0.238	0.356	0.452	0.193
*rs*10247238	0.325	0.675	0.334	0.666	0.050	0.550	0.400	0.101	0.466	0.432
*rs*726116	0.679	0.321	0.696	0.304	0.500	0.357	0.143	0.494	0.405	0.101
*rs*6969316	0.345	0.655	0.268	0.732	0.119	0.452	0.429	0.085	0.366	0.549
*rs*6947529	0.618	0.382	0.601	0.399	0.412	0.412	0.176	0.347	0.508	0.145
*rs*726805	0.65	0.35	0.569	0.431	0.425	0.450	0.125	0.340	0.458	0.201
*rs*2158768	0.439	0.561	0.453	0.547	0.244	0.390	0.366	0.220	0.467	0.313
*rs*1034805	0.682	0.318	0.662	0.338	0.471	0.424	0.106	0.427	0.471	0.102
*rs*2853552	0.69	0.31	0.735	0.265	0.476	0.429	0.095	0.565	0.340	0.095
*rs*10486329	0.488	0.512	0.447	0.553	0.222	0.531	0.247	0.211	0.474	0.316
*rs*17140399	0.354	0.646	0.384	0.616	0.083	0.542	0.375	0.152	0.464	0.384
*rs*7808451	0.606	0.394	0.552	0.448	0.388	0.435	0.176	0.316	0.471	0.213
*rs*17140423	0.671	0.329	0.706	0.294	0.512	0.317	0.171	0.520	0.372	0.108

*rs*6968777	0.394	0.606	0.4	0.6	0.176	0.435	0.388	0.173	0.453	0.373
*rs*2526633	0.286	0.714	0.298	0.702	0.071	0.429	0.500	0.122	0.353	0.526
*rs*8177374	0.833	0.167	0.832	0.168	0.708	0.250	0.042	0.698	0.268	0.034
*rs*6590224	0.293	0.707	0.173	0.827	0.098	0.390	0.512	0.065	0.216	0.719

Table 5: Initial Chr. 11 Allele and Genotype Frequencies

SNP	Cases - Alleles		Controls – Alleles		Cases - Genotypes			Controls - Genotypes		
	p	q	p	q	pp	pq	qq	pp	pq	qq
*rs*4938023	0.370	0.630	0.344	0.656	0.145	0.449	0.406	0.125	0.438	0.438
*rs*11214677	0.480	0.520	0.406	0.594	0.224	0.513	0.263	0.156	0.500	0.344
*rs*2587550	0.265	0.735	0.328	0.672	0.143	0.245	0.612	0.125	0.406	0.469
*rs*6276	0.743	0.257	0.625	0.375	0.579	0.329	0.092	0.406	0.438	0.156
*rs*2734839	0.286	0.714	0.636	0.364	0.286	0.000	0.714	0.364	0.545	0.091
*rs*4245147	0.517	0.483	0.450	0.550	0.283	0.467	0.250	0.200	0.500	0.300
*rs*4534613	0.395	0.605	0.419	0.581	0.186	0.419	0.395	0.194	0.452	0.355
*rs*12361261	0.917	0.083	0.833	0.167	0.905	0.024	0.071	0.667	0.333	0.000
*rs*7115090	0.566	0.434	0.466	0.534	0.344	0.443	0.213	0.379	0.172	0.448
*rs*4471464	0.448	0.552	0.484	0.516	0.194	0.507	0.299	0.194	0.581	0.226
*rs*1509513	0.567	0.433	0.556	0.444	0.467	0.200	0.333	0.296	0.519	0.185
*rs*12802646	0.348	0.652	0.348	0.652	0.152	0.394	0.455	0.121	0.455	0.424
*rs*7933647	0.688	0.313	0.500	0.500	0.500	0.375	0.125	0.000	1.000	0.000
*rs*11214716	0.850	0.150	0.859	0.141	0.767	0.167	0.067	0.719	0.281	0.000
*rs*2459976	0.563	0.438	0.652	0.348	0.292	0.542	0.166	0.485	0.333	0.182
*rs*6589396	0.677	0.323	0.883	0.117	0.484	0.387	0.129	0.767	0.233	0.000
*rs*1713676	0.480	0.520	0.310	0.690	0.280	0.400	0.320	0.000	0.621	0.379
*rs*7111825	0.677	0.323	0.667	0.333	0.507	0.338	0.154	0.467	0.400	0.133
*rs*11214741	0.453	0.547	0.362	0.638	0.209	0.488	0.302	0.138	0.448	0.414
*rs*2097078	0.275	0.725	0.150	0.850	0.101	0.348	0.551	0.033	0.233	0.733
*rs*6589400	0.223	0.777	0.188	0.813	0.089	0.268	0.643	0.000	0.375	0.625
*rs*1176746	0.730	0.270	0.547	0.453	0.514	0.432	0.054	0.281	0.531	0.188
*rs*17626940	0.145	0.855	0.214	0.786	0.048	0.194	0.758	0.071	0.286	0.643
*rs*238935	0.212	0.788	0.219	0.781	0.045	0.333	0.621	0.063	0.313	0.625
*rs*238914	0.271	0.729	0.355	0.645	0.063	0.417	0.521	0.129	0.452	0.419
*rs*238903	0.618	0.382	0.697	0.303	0.361	0.514	0.125	0.455	0.485	0.061
*rs*763857	0.353	0.647	0.359	0.641	0.133	0.440	0.427	0.094	0.531	0.375
*rs*1997547	0.125	0.875	0.318	0.682	0.063	0.125	0.813	0.091	0.455	0.455
*rs*2106234	0.721	0.279	0.594	0.406	0.557	0.328	0.115	0.344	0.500	0.156
*rs*648181	0.708	0.292	0.632	0.368	0.583	0.250	0.167	0.474	0.316	0.211
*rs*655988	0.256	0.744	0.194	0.806	0.103	0.308	0.590	0.032	0.323	0.645
*rs*371267	0.700	0.300	0.717	0.283	0.550	0.300	0.150	0.600	0.233	0.167
*rs*612592	0.772	0.228	0.875	0.125	0.603	0.338	0.059	0.750	0.250	0.000
*rs*676982	0.732	0.268	0.576	0.424	0.536	0.391	0.072	0.364	0.424	0.212
*rs*624590	0.167	0.833	0.422	0.578	0.061	0.212	0.723	0.219	0.406	0.375
*rs*695077	0.230	0.770	0.100	0.900	0.060	0.340	0.600	0.000	0.200	0.800
*rs*11220409	0.550	0.450	0.367	0.633	0.400	0.300	0.300	0.200	0.330	0.467
*rs*17656	0.783	0.217	0.925	0.075	0.633	0.300	0.067	0.850	0.150	0.000
*rs*499205	0.791	0.209	0.773	0.227	0.622	0.338	0.041	0.606	0.333	0.061
*rs*638766	0.805	0.195	0.578	0.422	0.661	0.288	0.051	0.375	0.406	0.219
*rs*667627	0.754	0.246	0.790	0.210	0.667	0.175	0.158	0.774	0.032	0.194
*rs*591163	0.307	0.693	0.500	0.500	0.040	0.533	0.427	0.286	0.429	0.286

*rs*8177376	0.809	0.191	0.879	0.121	0.640	0.337	0.022	0.788	0.182	0.030
*rs*1786704	0.195	0.805	0.258	0.742	0.035	0.319	0.646	0.091	0.333	0.576
*rs*648710	0.313	0.688	0.442	0.558	0.300	0.025	0.675	0.423	0.038	0.538
*rs*586566	0.760	0.240	0.864	0.136	0.584	0.351	0.065	0.788	0.152	0.061
*rs*240537	0.744	0.256	0.855	0.145	0.526	0.436	0.038	0.742	0.226	0.032
*rs*7937122	0.566	0.434	0.606	0.394	0.302	0.528	0.170	0.364	0.485	0.151
*rs*11220473	0.557	0.443	0.552	0.448	0.314	0.486	0.200	0.241	0.621	0.138
*rs*582037	0.332	0.668	0.394	0.606	0.109	0.446	0.446	0.212	0.364	0.424
*rs*2230279	0.731	0.269	0.766	0.234	0.500	0.463	0.037	0.531	0.469	0.000
*rs*752806	0.392	0.608	0.597	0.403	0.353	0.078	0.569	0.323	0.548	0.129
*rs*7925175	0.648	0.352	0.650	0.350	0.444	0.407	0.148	0.433	0.433	0.133
*rs*1943528	0.333	0.667	0.297	0.703	0.140	0.386	0.474	0.156	0.281	0.563
*rs*9971527	0.786	0.214	0.636	0.364	0.629	0.314	0.057	0.424	0.424	0.152
*rs*612841	0.434	0.566	0.375	0.625	0.224	0.421	0.355	0.094	0.563	0.344
*rs*625496	0.591	0.409	0.578	0.422	0.364	0.455	0.182	0.313	0.531	0.156
*rs*6590224	0.293	0.707	0.173	0.827	0.098	0.390	0.512	0.065	0.216	0.719
*rs*4935987	0.375	0.625	0.443	0.557	0.150	0.450	0.400	0.171	0.543	0.286
*rs*10893564	0.913	0.087	0.859	0.141	0.865	0.096	0.038	0.750	0.219	0.031
*rs*7127398	0.707	0.293	0.629	0.371	0.537	0.341	0.122	0.323	0.613	0.065
*rs*1946050	0.667	0.333	0.667	0.333	0.545	0.242	0.212	0.500	0.333	0.167
*rs*11220621	0.388	0.612	0.333	0.667	0.190	0.397	0.414	0.091	0.485	0.424
*rs*1106804	0.650	0.350	0.726	0.274	0.467	0.367	0.167	0.484	0.484	0.032
*rs*7110377	0.462	0.538	0.150	0.850	0.462	0.000	0.538	0.000	0.300	0.700
*rs*10750355	0.394	0.606	0.333	0.667	0.242	0.303	0.455	0.091	0.485	0.424
*rs*10893604	0.375	0.625	0.453	0.547	0.167	0.417	0.417	0.188	0.531	0.281
*rs*1628588	0.451	0.549	0.409	0.591	0.225	0.451	0.324	0.152	0.515	0.333
*rs*1793668	0.363	0.637	0.172	0.683	0.290	0.145	0.565	0.069	0.207	0.724
*rs*2508557	0.585	0.415	0.438	0.563	0.377	0.415	0.208	0.281	0.313	0.406
*rs*7941136	0.833	0.167	0.656	0.344	0.686	0.294	0.020	0.406	0.500	0.094

Table 6: Chr. 7 Allele and Genotype Frequencies of Validated SNPs

SNP	Cases - Alleles		Controls – Alleles		Cases - Genotypes			Controls - Genotypes		
	p	q	p	q	pp	pq	qq	pp	pq	qq
*rs*2237298	0.267	0.733	0.295	0.705	0.107	0.320	0.573	0.118	0.354	0.528
*rs*2158041	0.262	0.738	0.163	0.837	0.064	0.397	0.539	0.030	0.267	0.704
*rs*801759	0.130	0.870	0.045	0.955	0.021	0.218	0.761	0.000	0.091	0.909
*rs*801763	0.841	0.159	0.866	0.134	0.713	0.256	0.031	0.752	0.228	0.020
*rs*3807917	0.504	0.496	0.594	0.406	0.287	0.435	0.278	0.326	0.536	0.138
*rs*2520458	0.659	0.341	0.642	0.358	0.472	0.375	0.153	0.372	0.540	0.088
*rs*2695029	0.388	0.612	0.469	0.531	0.172	0.433	0.396	0.221	0.496	0.282
*rs*3213615	0.685	0.315	0.703	0.297	0.501	0.353	0.138	0.479	0.455	0.066
*rs*6974011	0.417	0.583	0.307	0.693	0.231	0.372	0.397	0.102	0.409	0.488
*rs*12699991	0.599	0.401	0.603	0.397	0.420	0.358	0.222	0.346	0.515	0.140

Table 7: Chr. 11 Allele and Genotype Frequencies of Validated SNPs

SNP	Cases - Alleles		Controls – Alleles		Cases - Genotypes			Controls - Genotypes		
	p	q	p	q	pp	pq	qq	pp	pq	qq
*rs*2587550	0.293	0.707	0.298	0.702	0.140	0.306	0.554	0.078	0.440	0.482
*rs*2734839	0.474	0.526	0.623	0.377	0.394	0.161	0.445	0.405	0.436	0.160
*rs*12361261	0.895	0.105	0.878	0.122	0.887	0.016	0.097	0.866	0.025	0.109
*rs*1509513	0.654	0.346	0.571	0.429	0.453	0.402	0.145	0.310	0.524	0.167
*rs*1713676	0.493	0.507	0.485	0.515	0.289	0.409	0.302	0.226	0.518	0.256
*rs*1176746	0.712	0.288	0.610	0.390	0.508	0.409	0.083	0.387	0.448	0.166
*rs*676982	0.735	0.265	0.726	0.274	0.566	0.337	0.096	0.560	0.333	0.107
*rs*624590	0.236	0.764	0.273	0.727	0.100	0.271	0.629	0.103	0.339	0.558
*rs*11220409	0.364	0.636	0.245	0.755	0.145	0.436	0.418	0.051	0.387	0.562
*rs*638766	0.763	0.238	0.723	0.277	0.619	0.288	0.094	0.554	0.339	0.107
*rs*591163	0.340	0.660	0.294	0.706	0.132	0.417	0.451	0.127	0.335	0.538
*rs*586566	0.749	0.251	0.780	0.220	0.582	0.333	0.085	0.617	0.326	0.057
*rs*240537	0.721	0.279	0.760	0.240	0.517	0.408	0.075	0.580	0.361	0.059
*rs*752806	0.461	0.539	0.544	0.456	0.270	0.382	0.349	0.302	0.483	0.215
*rs*6590224	0.243	0.757	0.159	0.841	0.071	0.343	0.586	0.03	0.26	0.711
*rs*10893564	0.866	0.134	0.834	0.166	0.791	0.149	0.060	0.692	0.284	0.024
*rs*7127398	0.705	0.295	0.629	0.371	0.538	0.333	0.128	0.323	0.613	0.065
*rs*1793668	0.366	0.634	0.285	0.715	0.189	0.354	0.457	0.085	0.400	0.515
*rs*2508557	0.593	0.407	0.520	0.480	0.360	0.467	0.173	0.295	0.451	0.254
*rs*7941136	0.813	0.187	0.671	0.329	0.655	0.317	0.029	0.616	0.321	0.063